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Metronomic S-1 Chemotherapy and Vandetanib: An Efficacious and Nontoxic Treatment for Hepatocellular Carcinoma[1]

Abstract

BACKGROUND: Metronomic chemotherapy involves frequent, regular administration of cytotoxic drugs at nontoxic doses, usually without prolonged breaks. We investigated the therapeutic efficacies of metronomic S-1, an oral 5-fluorouracil prodrug, and vandetanib, an epidermal growth factor receptor and vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitor, in models of hepatocellular carcinoma (HCC). *METHODS:* We compared anti-HCC effects and toxicity in the six treatment groups: control (untreated), maximum tolerated dose (MTD) S-1, metronomic S-1, vandetanib, MTD S-1 with vandetanib, and metronomic S-1 with vandetanib. Tumor microvessel density (MVD) and tumor apoptosis were evaluated by immunohistochemistry. The expression of VEGF and thrombospondin-1, an endogenous inhibitor of angiogenesis, was analyzed by Western blot. *RESULTS:* Metronomic S-1 significantly inhibited tumor growth, which was enhanced by combination with vandetanib. With respect to toxicities, MTD S-1 caused severe body weight loss and myelosuppression, whereas metronomic S-1 did not cause any overt toxicities. Moreover, metronomic S-1 or metronomic S-1 with vandetanib prolonged survival, the latter treatment providing the greatest benefit. Metronomic S-1 and metronomic S-1 with vandetanib decreased MVDs and increased apoptosis in tumor tissues. The expression of VEGF in tumor tissues was upregulated by vandetanib and metronomic S-1 with vandetanib, whereas the expression of thrombospondin-1 was upregulated by metronomic S-1 and metronomic S-1 with vandetanib. *CONCLUSION:* Metronomic S-1 with an antiangiogenic agent seems to be an effective and safe therapeutic strategy for HCC.

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common solid tumor and the third leading cause of cancer-related deaths globally [1]. Although prognosis of early and intermediate stage HCC has improved owing to advances in treatments, there are few proven effective systemic therapies for advanced HCC [2]. In particular, conventional chemotherapy using cytotoxic drugs for advanced HCC has not been shown to improve survival. Almost all cases of HCC occur in patients with chronic liver disorders, such as liver cirrhosis. Patients with liver cirrhosis have liver dysfunction and also pancytopenia. These pathologies

Abbreviations: HCC, hepatocellular carcinoma; MTD, maximum tolerated dose; MVD, microvessel density; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; TSP-1, thrombospondin-1; HUVEC, human umbilical vascular endothelial cell

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limit the use of conventional chemotherapy as a treatment strategy for HCC.

Conventional chemotherapy often involves pulsatile administration schedules using maximum tolerated doses (MTDs) of cytotoxic drugs. The long break periods between therapies not only allow recovery from various toxicities, especially myelosuppression, but also provide an opportunity, unfortunately, for the drug-treated tumors to recover as well [3]. In contrast, metronomic chemotherapy is given at frequent intervals using minimally or nontoxic doses without prolonged breaks. In several preclinical studies, such metronomic protocols have shown surprisingly effective antitumor effects, despite the reduced toxicity [4–6].

S-1 is an orally novel cytotoxic 5-flurouracil (5-FU) prodrug, which consists of tegafur and two biochemical modulators, 5-chloro-2,4-dihydroxypyridine and potassium oxonate [7]. 5-Chloro-2,4-dihydroxypyridine competitively inhibits dihydropyrimidine dehydrogenase approximately 180 times more effectively than uracil. Thus, S-1 gives rise to high concentrations of 5-FU in blood and tumor tissue for long-term periods since biochemical modulation [7,8]. A drug similar to S-1, namely, UFT, has been used successfully in metronomic preclinical studies [5]. Moreover, in the clinic it has been used successfully in randomized phase 3 trials in a metronomic fashion to treat in an adjuvant manner a variety of early stage cancers, after surgery, including non–small cell lung cancer [9] and breast cancer [10]. Because S-1 is thought to be more potent than UFT with respect to the effect of biochemical modulations, one might expect a stronger antitumor effect by using S-1 [7]. In this study, we describe a method of administering metronomic S-1 to treat HCC and compare it to conventional MTD S-1 chemotherapy, either alone or with an antiangiogenic drug.

Tyrosine kinase inhibitors such as sorafenib have proven activity in HCC patients and now represent one of the few effective systemic therapies for HCC [11]. Preclinical studies have also shown that the antitumor effect of metronomic chemotherapy can be significantly enhanced by combination with vascular endothelial growth factor (VEGF) pathway targeting agents [12,13]. In this study, we show here that metronomic S-1 might be a promising therapy to consider for concurrent daily combination with an oral antiangiogenic drug, in this case, vandetanib (ZD6474; AstraZeneca Pharmaceuticals, Macclesfield, UK). Vandetanib inhibits not only the catalytic function of VEGFR-2 but also EGF receptors (EGFRs), in contrast to sorafenib or sunitinib that do not affect EGFRs [14]. We evaluated the efficacies of vandetanib alone *in vivo* for HCC-bearing mice using various hepatoma cell lines that had different expressions of EGFR (submitted for publication). EGFR is known to contribute to 5-FU drug resistance, and 5-FU is the major metabolite of S-1 [15]. Therefore, there is a rationale for drug targeting of both EGF receptors and VEGF receptors along with metronomic chemotherapy, which was the purpose of this study. Thus, we investigated the efficacy of combining with each treatment schedule of S-1 and vandetanib using two HCC cell lines, which express low or high levels of EGFR, that is, KYN-2 and Huh-7, respectively. Overall, our results suggest that the combination treatment of metronomic S-1 plus vandetanib may be useful for the therapy of HCC.

Materials and Methods

Cell Lines and Culture

In human hepatoma cell lines, Huh-7 was originally purchased from CAMBREX Bio Science Walkersville, Inc (Walkersville, MD), and KYN-2 was provided by the Department of Pathology, Kurume University School of Medicine. Cells were maintained in Dulbecco modified Eagle medium (DMEM; Gibco Invitrogen Cell Culture Co, Auckland, New Zealand) supplemented with 10% fetal bovine serum (FBS).

Human umbilical vascular endothelial cells (HUVECs) were purchased from CAMBREX Bio Science Walkersville, Inc, and maintained with endothelial cell growth medium-2 (Clonetics, San Diego, CA) containing 5% FBS.

Animals and Drugs

Male 5-week-old nude mice (BALB/c *nu/nu*) were purchased from Kyudo KK (Fukuoka, Japan). All experiments were conducted in accordance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals.

5-FU was purchased from Kyowa Hakko Kogyo Co, Ltd (Tokyo, Japan). S-1 was provided by Taiho Pharmaceutical Co, Ltd (Tokyo, Japan). S-1 consists of a mixture of tegafur, gimeracil, and oteracil at molar ratio of 1:0.4:1 in 0.5% hydroxypropylmethylcellulose (HPMC) solutions. Vandetanib (ZD6474; Zactima) was provided by AstraZeneca Pharmaceuticals (Macclesfield, UK).

In Vitro *Cell Proliferation Assay*

As the tegafur component of S-1 is physiologically converted to 5-FU in the body, we evaluated the difference of antiproliferative effects *in vitro* of 5-FU using different schedules with both hepatoma cells and HUVECs. Approximately 1000 cells in 100 μl of DMEM containing 10% FBS was added to each well of 96-well plate. After incubation for 24 hours, the medium was exchanged to the serum-containing medium with various concentrations of 5-FU (0, 1, 10, 100, 500, 1000, 10,000 ng/ml). Each cell line was exposed to 5-FU for 5 days. To evaluate the antiproliferative effect of "MTD" *versus* "metronomic" chemotherapy, exchange of the medium containing 5-FU was performed using different schedules. For the metronomic schedule, the medium containing 5-FU was exchanged daily as described previously [16]. For the MTD schedule, the medium containing 5-FU was not changed. After incubation, cell proliferation was evaluated by a tetrazolium-based assay (Cell Count Reagent SF; Nakalai Tesque, Inc, Kyoto, Japan).

Determination of the Optimal Dose for S-1 Using Metronomic Chemotherapy

We determined the optimal metronomic dose of S-1 according to a previous report, which involved evaluating different doses of a chemotherapy drug both for antitumor effects and toxicity, with the aim of determining a dose that has minimal toxicity but retains good efficacy [17]. A total of 5×10^6 Huh-7 cells were injected into the flank regions of nude mice. Therapy with different doses of S-1 was initiated when the estimated tumor volume ($0.52 \times \text{length} \times \text{width}^2$) reached 150 to 200 mm^3. Mice received S-1 orally administrated by gavage with the following agents on a daily basis for 14 days: 1) HPMC as the control group; 2) S-1, 7.5 mg/kg per day; 3) S-1, 5.0 mg/kg per day; 4) S-1, 2.5 mg/kg per day; or 5) S-1, 1.0 mg/kg per day. Tumor-bearing mice were randomly divided into groups of 10 mice. The mice were killed at day 15 after start of treatment. The inhibition rate of tumor growth (IR %) was calculated as follows: IR % = (1 – mean RTV of treatment group / mean RTV of control group) × 100, where RTV indicates the relative tumor volume: tumor volume on killing / tumor volume on initial treatment. For comparison of the toxicity in each group, mouse body weights were measured every 3 days. Peripheral leukocyte count and hemoglobin (Hb) concentrations of these mice were also measured at day 15.

Tumor Growth and Toxicity Assessment in the Subcutaneous Tumor Transplant Model

We selected as the optimal metronomic dosage for S-1, 5.0 mg/kg per day based on our aforementioned study. We selected the MTD for S-1 15 mg/kg per day for 7 days, followed by a 7-day break period, based on previous published findings [6]. To compare the antitumor effect and toxicity caused by MTD or metronomic S-1, long-term experiments were performed using the Huh-7 subcutaneous transplant model. Mice were randomly divided to six groups: 1) HPMC as the control group; 2) MTD S-1, 15 mg/kg per day p.o. for 1 week, followed by a 1-week break period for a cumulative dose of 95 mg/kg; 3) metronomic S-1, 5 mg/kg per day p.o. for 2 weeks without any break period for a cumulative dose of 70 mg/kg; 4) vandetanib 25 mg/kg per day p.o. for 2 weeks; 5) MTD S-1 with vandetanib; or 6) metronomic S-1 with vandetanib. Each group consisted of 10 mice. It is important to note that the cumulative metronomic doses were distinctly less than the cumulative MTD. In other words, whereas the schedule used was "dose-dense," it was not "dose intense." The aforementioned schedules were performed in two cycles, 4 weeks in total. Estimated tumor volumes were measured every 3 days, and all mice were killed after 4 weeks of treatment. For comparison of the toxicity in each group, mouse body weights were measured every 3 days. Peripheral leukocyte count and hemoglobin (Hb) concentrations in these mice were also measured at sacrifice.

Tumor Growth and Survival Assessment in the Orthotopic Transplant Model

We also examined tumor growth using an orthotopic liver transplant model. The mice were implanted with 2×10^6 KYN-2 cells into the left lobe liver. Mice were randomly divided into six groups, as outlined above, and therapy was initiated 7 days after implantation of tumor cells. Each group consisted of 10 mice. The mice were killed at day 29 of initial treatment, and tumor volumes were evaluated.

In addition, a survival study was also performed using the KYN-2 orthotopic transplant model for the six groups as mentioned above. Each group consisted of 10 mice. In the group for survival observation, animals were killed according to (pre)clinical signs of weakness, for example, anorexia, or greater than 20% weight loss, and days of life were recorded from initial treatment.

Immunohistochemical Staining of CD31, PCNA, and TUNEL

The sections of all tumor tissues obtained from KYN-2 orthotopic transplant model were boiled for 30 minutes by high pH target retrieval solution (DAKO Japan, Kyoto, Japan) for antigen retrieval. The sections were incubated with rabbit anti–human CD31 antibody (diluted 1:300; Abcam, Inc, Tokyo, Japan) and rabbit anti–human PCNA antibody (diluted 1:250; Abcam, Inc) at 4°C overnight. Then the avidin-biotin procedures were subsequently performed using a Vectastatin ABC Kit (Vector Laboratories, Inc, Burlingame, CA). The sections were reacted with 0.005% H_2O_2–3,3′-diaminobenzidine at room temperature for 1 minute. For quantification of microvessel density (MVD), CD31-positive vessels were counted in randomly selected 30 areas per five tumors in each treatment group at 200-fold magnification.

The terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) method was performed for the evaluation of apoptosis in each of the treated tumor tissues. TUNEL labeling was performed using the ApopTag Kit (Chemicon, Temecula, CA) according to the manufacturer's instructions. The stained sections of tumors of each group were reviewed, and the apoptosis index, determined by TUNEL staining, was determined by counting at least 1000 cells in five randomly selected high-power fields (magnification, ×200).

Expression of Thrombospondin-1 and VEGF in Tumor Tissues

We examined the expression of VEGF and thrombospondin-1 (TSP-1) in treated tumor tissues using Western blot analysis. TSP-1 is a known endogenous antiangiogenic protein [18]. Five samples of each treatment group and control group were loaded in equal conditions, respectively. Thirty micrograms of protein was loaded onto a NuPAGE 4% to 12% Bis-Tris gel (Invitrogen, CA). Membranes were incubated with rabbit anti–TSP-1 antibody (1:350 dilution; Abcam, Inc) or rabbit anti-VEGF antibody (1:500 dilution; Abcam, Inc) at 4°C overnight. Equal protein loading was assessed by mouse anti–β-actin antibody (1:1000 dilution; Sigma, St Louis, MO). After incubation with HRP-conjugated anti–rabbit immunoglobulin G (1:10,000 dilution; GE Healthcare UK Ltd, Buckinghamshire, UK) or HRP-conjugated anti–mouse immunoglobulin G antibody (1:5000 dilution; GE Healthcare UK Ltd) for 1 hour, immunoreactive bands were stained by an enhanced chemiluminescence Western blot analysis system using LAS 4000 mini (Fujifilm, Tokyo, Japan) and were calculated with the amount of luminescence in each sample using multigauge software (Fujifilm). The relative amount of luminescence in each treatment group for the control group was expressed as [(treatment group VEGF or TSP-1 / treatment group β-actin) / (control group VEGF or TSP-1 / control group β-actin)] and compared with each group.

Statistical Analysis

All experimental data were expressed as mean ± SD. Differences between groups were examined for statistical significance using the Mann-Whitney *U* test, the Kruskal-Wallis test, and nonparametric analysis of variance. If the one-way analysis of variance was significant, differences between individual groups were estimated using the Fisher least significant difference test. Overall survival was estimated according to the Kaplan-Meier method and compared using the log-rank test. $P < .05$ was considered to be statistically significant.

Results

Comparison of Antiproliferative Effects of Metronomic versus MTD Type Chemotherapy In Vitro

The 50% inhibitory concentration (IC_{50}) levels of metronomic and MTD schedules of 5-FU, the major metabolite of S-1, for each cell line are shown in Table 1. The antiproliferative effects of 5-FU for each cell line were found to be dose-dependent (Figure 1, *A–C*). The IC_{50} levels for the MTD and metronomic schedule for Huh-7 cells were 3.84 and 0.77 μM, respectively (Figure 1*A*). The IC_{50}

Table 1. IC_{50} Levels of MTD and Metronomic Schedule in Hepatoma Cell Lines and Endothelial Cell.

	5-FU IC_{50} (μM)	
	MTD	Metronomic
Hepatoma cell lines		
Huh-7	3.84	0.77
KYN-2	7.69	3.84
Endothelial cell		
HUVECs	7.7	0.76

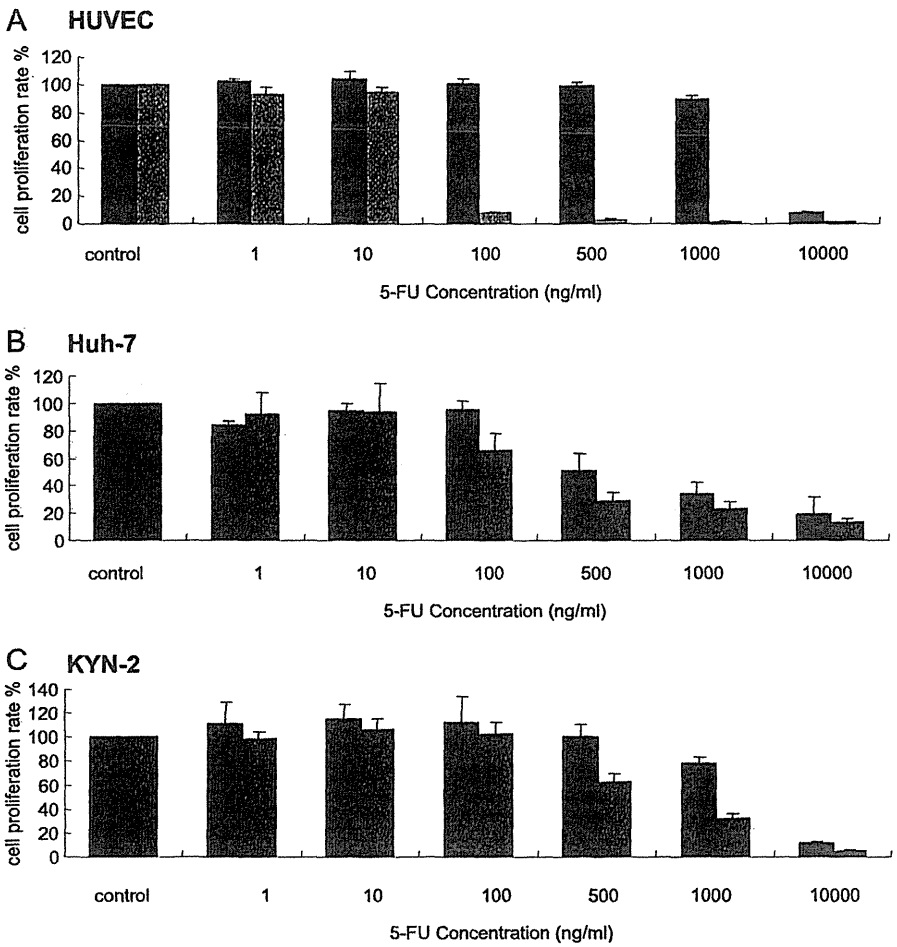


Figure 1. Inhibitory effect of metronomic chemotherapy for each cell line tested in a cell proliferation assay. To evaluate the antiproliferative effect of "MTD" and "metronomic" chemotherapy *in vitro*, exchange of the medium containing 5-FU was performed in different schedules. For the metronomic schedule, the medium containing 5-FU (0, 1, 10, 100, 500, 1000, and 10,000 ng/ml) was exchanged once a day. For the MTD schedule, the medium containing 5-FU was not changed. Data are shown as a ratio of the control and expressed as mean ± SD of 10 samples. *$P < .001$ compared with each schedule. Dark gray–shaded columns show MTD schedule, and light gray–shaded columns showed metronomic schedule. (A) HUVEC. HUVEC was cultured with 100 μl of endothelial cell growth medium-2 with 5% FBS containing 5-FU. (B) Huh-7. (C) KYN-2. Hepatoma cells were cultured with 100 μl of DMEM with 10% FBS containing 5-FU.

levels for KYN-2 were 7.69 and 3.84 μM, respectively. For the hepatoma cell lines, the metronomic schedule inhibited cell proliferation at approximately 1/2 to 1/4 concentrations of 5-FU compared with MTD schedule (Table 1). The metronomic schedule for HUVECs inhibited cell proliferation at apparently lower levels (IC_{50} levels, 0.76 μM) approximately 1/10 the concentration of 5-FU compared with MTD schedule (IC_{50} levels, 7.7 μM; Table 1).

Determination of the Optimal Dose of S-1 for Metronomic Chemotherapy In Vivo*: Maximum Tumor Growth Inhibition with Minimal Toxicity*

In the 7.5- and 5.0-mg/kg-per-day S-1 treatment groups, there were significant differences in suppression of tumor growth compared with the control group ($P < .05$; Figure 2*A*), and dosages lower than 2.5 mg/kg per day S-1 were not statistically effective compared with the control group. In addition, we evaluated body weight loss and myelosuppression toxicities associated with administration of S-1 (Figure 2, *B–D*). With respect to body weight loss, there was no significant difference between each group (Figure 2*B*). But only the 7.5-mg/kg-per-day group showed severe toxicity as determined by reductions in Hb concentration and leukocyte count ($P < .001$, compared with the control group; Figure 2, *C* and *D*). Therefore, we selected 5.0 mg/kg per day as the optimal metronomic dosage of S-1, which was used in all subsequent experiments.

Evaluation of the Antitumor Effect and Toxicity for Metronomic S-1 Chemotherapy in the Subcutaneous Transplant Tumor Model

In the assay for tumor growth, statistical differences were observed between the control group and all treatment groups (Figure 3*A*).

Metronomic S-1 potently inhibited tumor growth compared with MTD S-1 ($P < .01$). The mean tumor volumes were 4810.5 ± 1440.9 cm^3 in the control group, 3212.6 ± 1364.7 cm^3 in the MTD S-1 group, 1927.1 ± 652.9 cm^3 in the metronomic S-1 group, and 2331.4 ± 662.1 cm^3 in the vandetanib group, respectively. The mean tumor volumes in the MTD S-1 plus vandetanib group and metronomic S-1 plus vandetanib group were 2026.7 ± 1106.7 and 1383.7 ± 697.5 cm^3, respectively. The greatest inhibition of tumor growth was induced by the metronomic S-1 in combination with vandetanib (Figure 3*A*). In addition, we evaluated toxicity in each of Huh-7 subcutaneous tumor treatment groups (Figure 3, *B–D*). In leukocyte count, there were no significant differences in the groups (Figure 3*B*). In Hb concentration, the control group was 12.84 ± 1.82 g/dl, the MTD S-1 group was 9.77 ± 3.63 g/dl, the metronomic S-1 group was 11.73 ± 3.27 g/dl, and the vandetanib group was 12.34 ± 2.77 g/dl. For the combination treatments, the MTD S-1 plus vandetanib group was 8.24 ± 1.64 g/dl, and for the metronomic S-1 plus vandetanib group, it was 11.74 ± 1.55 g/dl (Figure 3*C*). With respect to rate of body weight loss, in the MTD S-1 monotherapy and MTD S-1 with vandetanib groups, the values observed were 10.48% ± 6.85% and 8.59% ± 5.02% reduction compared with the control group, respectively. Vandetanib, metronomic S-1, and the combination therapy resulted in 5.64% ± 4.23%, 3.04% ± 2.23%, and −0.51% ± 5.56% reduction compared with the control group, respectively (Figure 3*D*). Both the MTD S-1 and MTD S-1 plus vandetanib treatment groups experienced severe body weight loss and reduced Hb concentrations compared with the control group (Figure 3, *C* and *D*). In marked contrast, the metronomic S-1 monotherapy and metronomic S-1 with vandetanib groups did not manifest any overt toxicity (Figure 3, *B–D*).

Evaluation of Antitumor Efficacy Using Metronomic S-1 Chemotherapy in an Orthotopic Liver Transplant Model

For tumor volume assessments, all treatments except MTD S-1 monotherapy were effective compared with the control group (Figure 4*A*). Tumor volumes at sacrifice were 4186.0 ± 1128.0 cm^3 in the control group, 3259.0 ± 788.7 cm^3 in the MTD S-1 group, 1501.3 ± 1002.2 cm^3 in the metronomic S-1 group, and 1582.0 ± 354.9 cm^3 in the vandetanib group. There was a significant difference between metronomic S-1 and MTD S-1 in tumor growth inhibition ($P < .05$; Figure 4*A*). For the combination treatment groups, tumor volumes were 931.1 ± 331.7 cm^3 in the MTD S-1 plus vandetanib group and 875.0 ± 369.4 cm^3 in the metronomic S-1 plus vandetanib group. There was no significant difference between the metronomic S-1 plus vandetanib group and the MTD S-1 plus vandetanib group. However, the greatest inhibition of tumor growth was detected in the metronomic S-1 plus vandetanib treatment group ($P < .001$; Figure 4*A*).

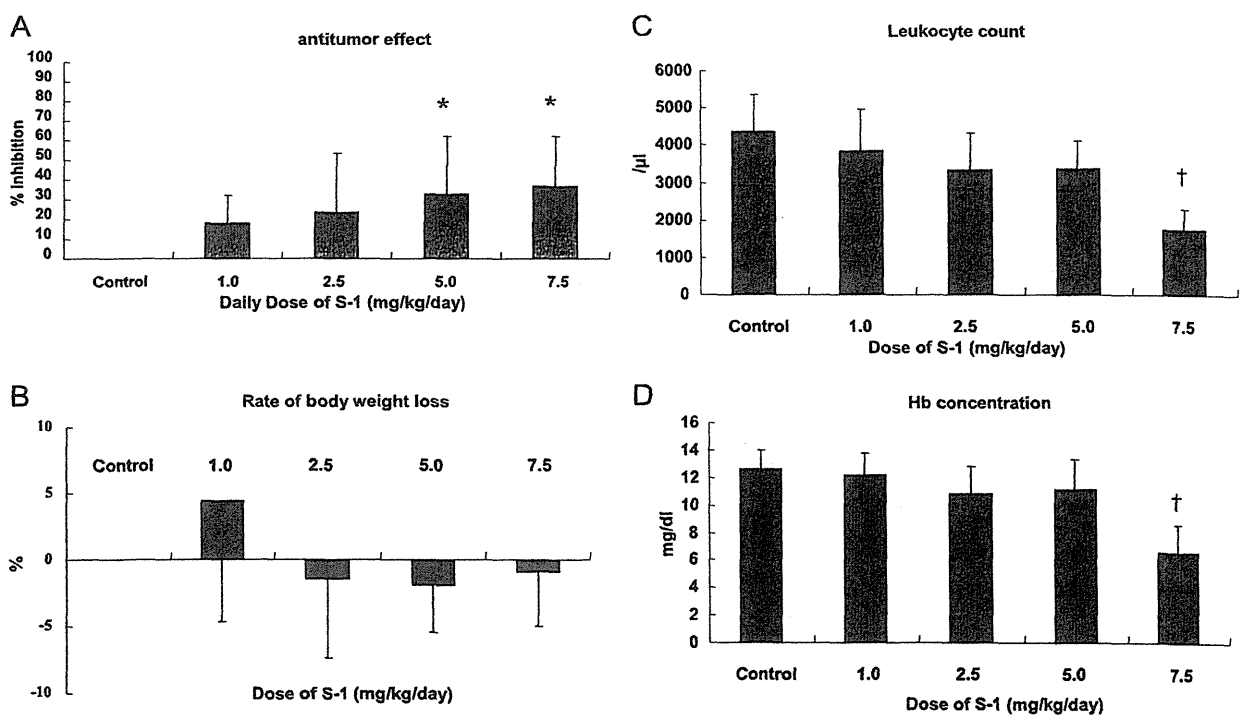


Figure 2. Determination of the optimal dose of S-1 in metronomic chemotherapy. Huh-7 subcutaneous tumor models were treated daily with either HPMC or different metronomic doses of S-1 (1.0, 2.5, 5.0, or 7.5 mg/kg per day) for 14 consecutive days. (A) Inhibition rates of tumor volumes (%) are expressed as mean ± SD ($n = 10$ per group). Dosages of 5.0 and 7.5 mg/kg per day S-1 groups statistically inhibited tumor growth compared with the control group (*$P < .05$). (B–D) Toxicity parameters are represented as mean ± SD. (B) Body weight (BW) changes on killing were calculated according to the following formula: BW change (%) = [(BW on sacrifice) − (BW on day 0)] × 100. (C) Hb concentration. (D) Leukocyte count. Each different dose of S-1 did not show body weight loss. However, the only 7.5-mg/kg-per-day S-1 group represented severe myelosuppression, such as decreased Hb concentration or leukocyte count. †$P < .001$ by compared with the control group.

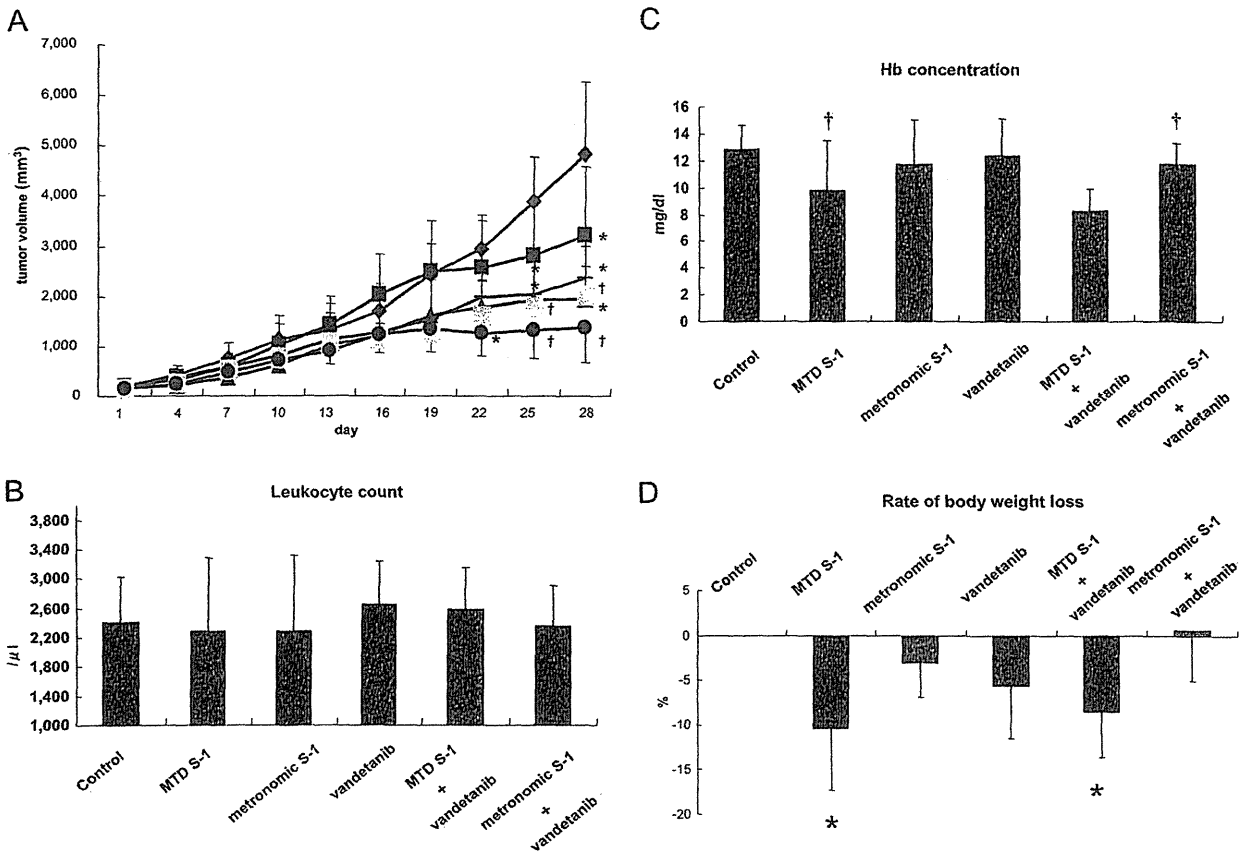


Figure 3. Therapeutic effects of metronomic S-1 chemotherapy in the Huh-7 subcutaneous tumor transplant model. (A) Tumor-bearing nude mice (n = 10 per group) were treated in the following six groups: 1) HPMC as the control group (blue); 2) MTD S-1 15 mg/kg per day for 1 week, followed by a 1-week break period (purple); 3) metronomic S-1 5 mg/kg per day for 2 weeks without break period (green); 4) vandetanib 25 mg/kg per day for 2 weeks (red); 5) MTD S-1 with vandetanib (yellow); or 6) metronomic S-1 with vandetanib (black). All treatments were performed for 4 weeks in total. Tumor volume changes are expressed as mean ± SD. All treatments showed efficacy compared with the control group (*P < .05), and the metronomic S-1 therapy was more effective than the MTD S-1 treatment. The metronomic S-1 with vandetanib significantly inhibited tumor growth compared with the control group (†P < .001). (B–D) Toxicity parameters are expressed as mean ± SD. (B) Hb concentration. (C) Leukocyte count. (D) Rate of body weight loss. MTD S-1 and the MTD S-1 with vandetanib showed severe body weight loss (*P < .01) and decreased Hb concentration (†P < .05) compared with the control group. Metronomic S-1 and the metronomic S-1 with vandetanib did not show any overt toxicities.

Evaluation of Survival Using Metronomic S-1 Chemotherapy in an Orthotopic Liver Transplant Model

The mean survival time in the control group was 28.9 ± 6.4 days. MTD S-1 did not prolong survival (mean survival time, 29.6 ± 3.9 days). In contrast, metronomic S-1 significantly prolonged survival (mean survival time, 34.3 ± 4.8 days). The mean survival time in the vandetanib group was 33.6 ± 5.0 days. MTD S-1 plus vandetanib treatment did not prolong survival times compared with vandetanib monotherapy (mean survival time, 37.6 ± 5.5 days). However, the metronomic S-1 plus vandetanib group provided the greatest prolonged survival times among all the treatment groups (mean survival time, 49.6 ± 11.5 days; Figure 4*B*).

Effect of Metronomic S-1 Chemotherapy Alone and in Combination with Vandetanib on Parameters of Tumor Angiogenesis

The results in Figure 5 show the MVD count in each treatment group. There was no significant difference in the MVD count between the control and the MTD S-1 group (control 41.1 ± 9.2, MTD S-1 35.8 ± 5.5; Figure 5*B*). However, tumor MVD was decreased in the metronomic S-1 group (17.2 ± 4.1) compared with the control group (P < .001) and the MTD S-1 group (P < .001; Figure 5*B*). Tumor MVD in mice treated with vandetanib was 13.7 ± 5.1. In the MTD S-1 plus vandetanib group, the MVD count was 18.8 ± 7.4. Metronomic S-1 plus vandetanib group showed the greatest reduction of tumor MVD (P < .01 compared with MTD S-1 plus vandetanib group, 8.2 ± 1.6; Figure 5*B*).

Detection of Proliferation and Apoptotic Cells in Tumor Tissues

To further investigate the mechanism of the observed antitumor effect, we examined the effect of metronomic S-1 and in combination with vandetanib on tumor cell proliferation and apoptosis (Figure 5). With respect to tumor cell proliferation, there were no differences between the control and all treated groups. The mean

number of apoptotic tumor cells (apoptotic index) measured in the control group was 6.2 ± 2.6. The MTD S-1 group did not show any significant difference (6.1 ± 4.9). However, the metronomic S-1 and vandetanib groups showed a significant increase in the apoptosis index (26.0 ± 5.4 and 18.4 ± 8.8, respectively, $P < .0001$). A significant increase in the tumor cell apoptosis index was also observed in the metronomic S-1 plus vandetanib group with 42 ± 3.5 ($P < .0001$).

Expression of VEGF and TSP-1 in Tumor Tissues

The results in Figure 6 show the expression of TSP-1 and VEGF in treated tumor tissues. The expression level of TSP-1 was significantly upregulated by approximately two- to three-fold in both the metronomic S-1 and the metronomic S-1 plus vandetanib treatment groups ($P < .05$ compared with the control group; Figure 6, *A* and *B*). With respect to expression levels of VEGF, there were no differences between the control and the MTD S-1 and metronomic S-1 groups (Figure 6, *C* and *D*). In contrast, the vandetanib and the metronomic S-1 plus vandetanib groups showed significantly upregulated the VEGF expression compared with the control group ($P < .05$; Figure 6, *C* and *D*). There was a significant difference between the vandetanib monotherapy group and the metronomic S-1 plus vandetanib treatment group ($P = .045$).

Discussion

Our results add to an expanding body of literature reporting the therapeutic benefit of metronomic chemotherapy, especially when it is combined concurrently with a targeted antiangiogenic drug [5,12,13]. Moreover, to our knowledge, this is the first preclinical report of using S-1 in a metronomic dosing and administration schedule for HCC preclinical model. Also noteworthy is that we undertook a comparative

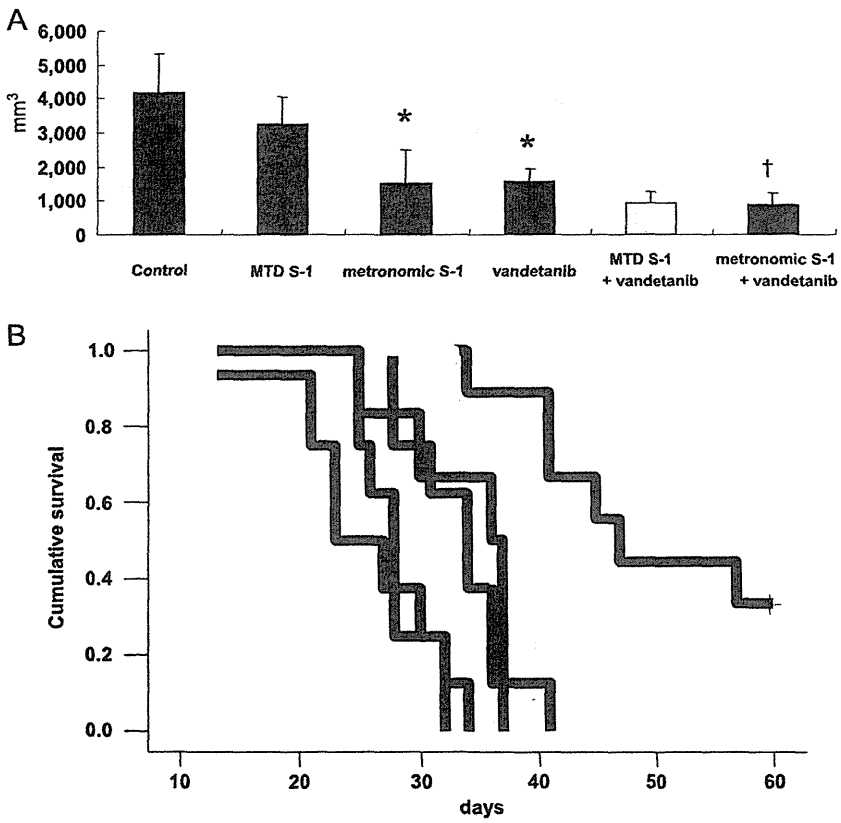
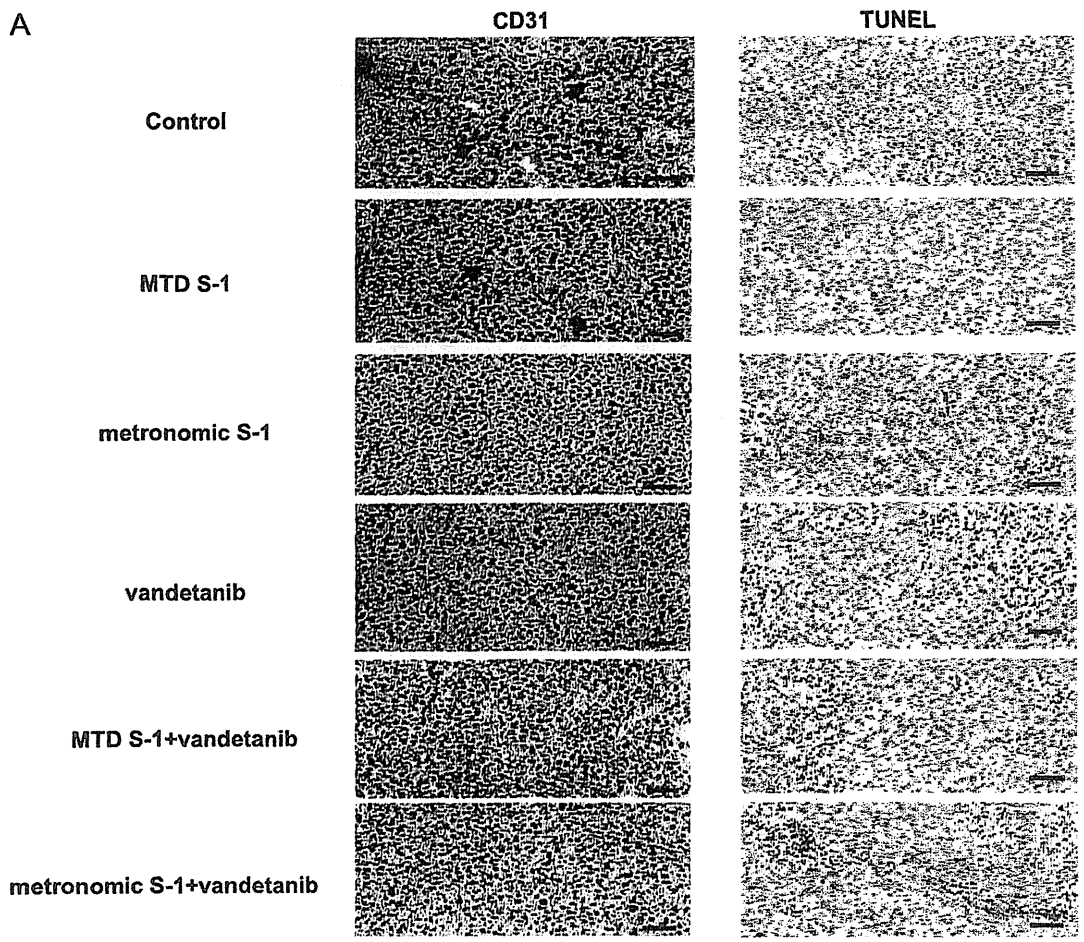


Figure 4. Assessment of therapeutic effects in KYN-2 liver transplant model. Tumor-bearing nude mice were treated in the following six groups: 1) HPMC as the control group (blue); 2) MTD S-1: 15 mg/kg per day for 1 week, followed by 1 week break period (purple); 3) metronomic S-1: 5 mg/kg per day for 2 weeks without break period (green); 4) vandetanib 25 mg/kg per day for 2 weeks (red); 5) MTD S-1 with vandetanib (yellow); or 6) metronomic S-1 with vandetanib (black). (A) Inhibition of tumor growth for KYN-2 liver transplant model. All treatments were performed 4 weeks in total. There was no significant difference between the control and the MTD S-1 groups. The metronomic S-1 group contributed to obvious inhibitory effect of tumor growth (*$P < .05$ compared with the control and the MTD S-1 groups). The metronomic S-1 with vandetanib treatment group showed the greatest inhibitory effect of tumor growth among all the groups (†$P < .001$). (B) Survival of mice treated with MTD S-1 or metronomic S-1 and in combination with vandetanib ($n = 10$ per group). Treatment was continued until mice were moribund, and days of life were recorded. Survival data were compared for significance with the log-rank test. MTD S-1 did not prolong survival compared with the control group. In contrast, metronomic S-1 prolonged survival compared with the control and MTD S-1 groups. The metronomic S-1 with vandetanib group provided the most effective therapy with longest survival times among all the groups ($P < .001$).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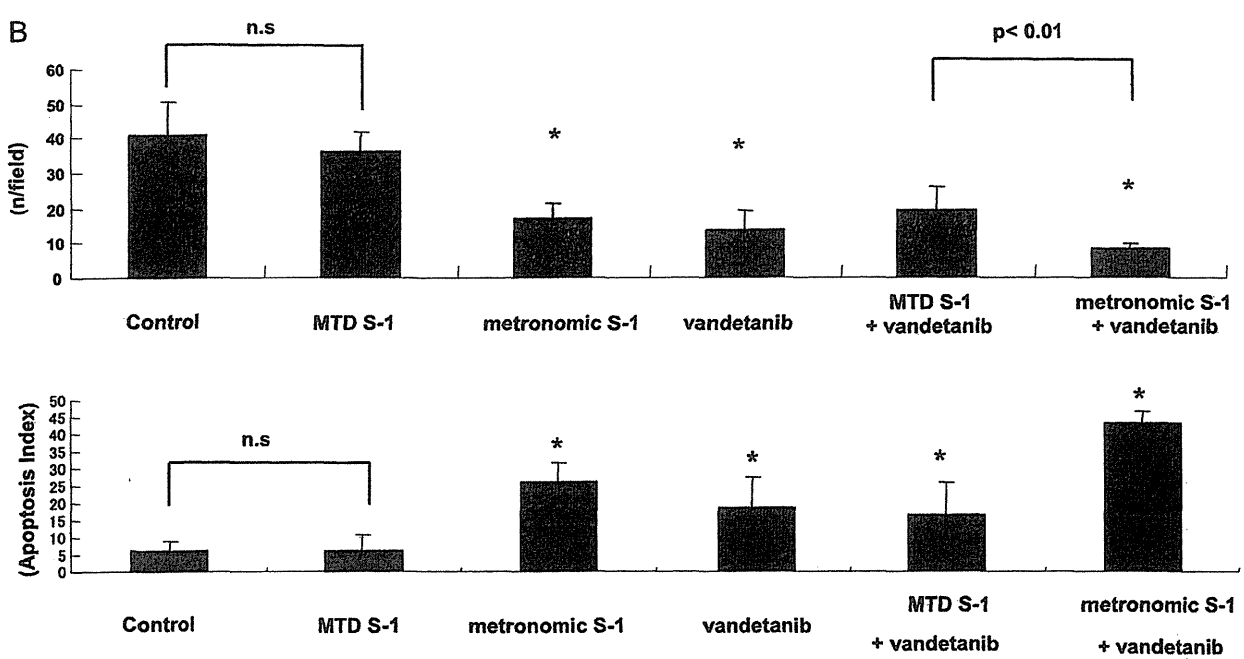
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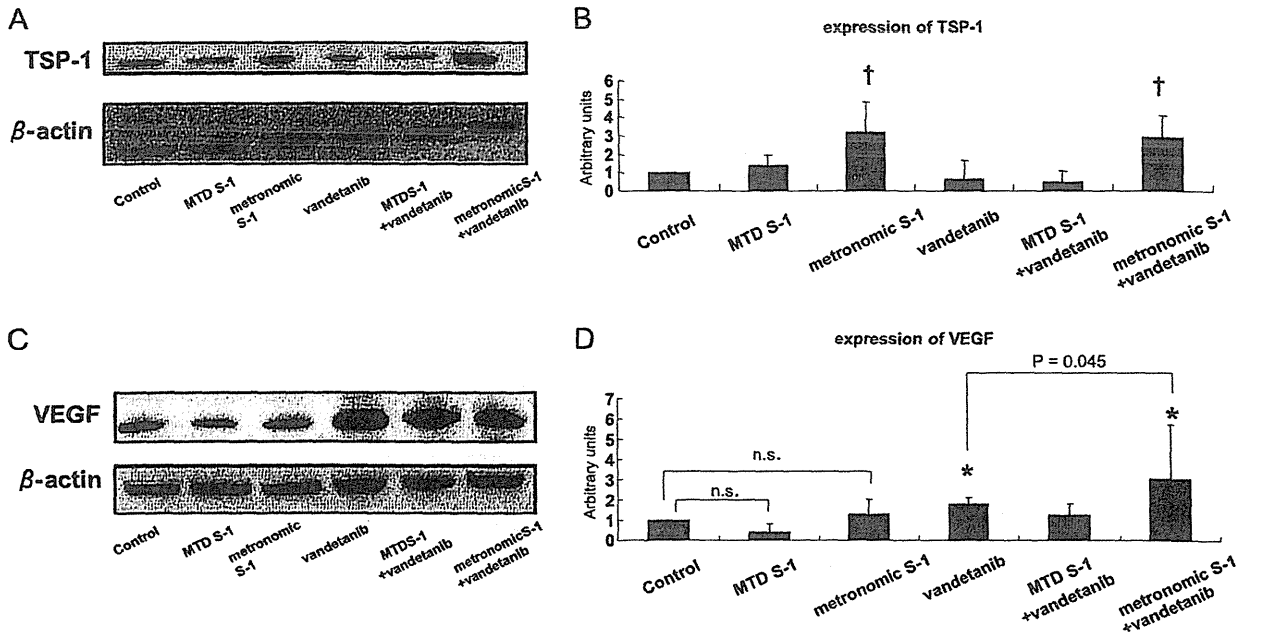

Figure 6. Western blot analysis of TSP-1 and VEGF. The band intensities of both TSP-1 and VEGF in treatment groups were measured and calibrated with each protein in control group and β-actin. (A and B) The metronomic S-1 and metronomic S-1 with vandetanib groups showed strongly upregulated the expression of TSP-1 ($^{\dagger}P < .001$ compared with the control group). (C and D) The expression of tumor VEGF was increased by the vandetanib and the metronomic S-1 with vandetanib groups. There were no differences between the control and the MTD S-1, metronomic S-1 group ($^{*}P < .05$ compared with the control group). There was a significant difference between the vandetanib and metronomic S-1 with vandetanib group ($P = .045$).

analysis of the effects of S-1 given in a more conventional MTD schedule with metronomic S-1, and our results consistently showed the metronomic dosing/schedule was superior to the MTD protocol, both in terms of increased antitumor efficacy and reduced toxicity. Importantly, in this regard, the metronomic protocol we used involved a cumulative dose over time that was 30% less than the corresponding MTD protocol. Below we discuss a number of different aspects of our results and some of the translational/clinical implications.

Antiangiogenic Effects Mediated by Metronomic S-1 Chemotherapy

Previous studies during the last decade have indicated that metronomic chemotherapy regimens using cytotoxic agents inhibit tumor growth by various mechanisms, namely, antiangiogenic effects, direct tumor cell targeting effects, or anticancer immune responses [4,19, 20]. Our results with metronomic S-1 would seem to confirm the antiangiogenic effect findings. First, we found that exposure of 5-FU in a metronomic-type protocol *in vitro* brought about a greater antiproliferative effect at distinctly low concentrations not only of 5-FU on two different tumor cell lines but also, especially, HUVECs, compared with an MTD-like exposure. This is similar to the results of other studies such as that of Bocci et al. [16] using paclitaxel or the active metabolite of cyclophosphamide. Second, we found reduced MVD and increased number of apoptotic tumor cells in mice treated with the metronomic S-1 schedule but not the MTD protocol. Third, we observed an increased expression of TSP-1, which has been reported previously using other cytotoxic drugs administered in a metronomic fashion *in vivo*, including cyclophosphamide [21]. Fourth, we noted that a tumor cell line (KYN-2) that is intrinsically resistant *in vitro* to high concentrations of 5-FU—the major metabolite of S-1—nevertheless responds to metronomic S-1 *in vivo* but not to MTD S-1, suggesting that a target other than the tumor cell population *per se* is likely involved in the *in vivo*

Figure 5. MVD and apoptosis in tumors tissues. The sections of tumors from the KYN-2 liver transplant model were stained by anti-CD31 antibody and Terminal deoxynucleotidyl transferase (TdT)–mediated dUTP nick-end labeling (TUNEL). Original magnification, ×200. The density of CD31-positive vessels (arrow) and TUNEL in a tumor field are represented as mean ± SD ($n = 30$ per group). (A) Representative sections for each treatment are shown. Bar, 10 μm. (B) There was no significant difference in MVD between the control and the MTD S-1 groups. Tumor vessel numbers were reduced by metronomic S-1. The metronomic S-1 with vandetanib group showed the most inhibitory effect of tumor vessel count among all the groups ($^{*}P < .001$ compared with the control group and the MTD S-1 group). The MTD S-1 group did not show any significant difference in the number of tumor cell apoptosis index (6.1 ± 4.9). However, the metronomic S-1 and vandetanib groups significantly increased in the number of apoptosis index, respectively (26.0 ± 5.4 and 18.4 ± 8.8, $P < .0001$). A significant increase of tumor cell apoptosis index was also observed in the metronomic S-1 with vandetanib group with 42 ± 3.5 ($P < .0001$).

antitumor activity that was observed using metronomic S-1. Fifth, O'Reilly et al. [22] have reported that the antiangiogenic effect mediated by endogenous antiangiogenic factors induces increased apoptosis of tumor cells, likely a secondary effect due to decreased MVD, whereas proliferation of tumor cells was not affected. Similarly, tumor apoptotic cell numbers were increased, whereas proliferation of tumor cells was not inhibited by metronomic S-1 chemotherapy in our study. On the basis of all of the aforementioned data and information, the antitumor effect of metronomic S-1 chemotherapy was likely to be mainly through antiangiogenesis mediated by inhibiting the proliferation of endothelial cells and inducing the expression of TSP-1, although some additional mechanisms cannot be entirely excluded. The mechanism of antiangiogenesis of metronomic S-1 chemotherapy is thought to be quite different from that of vandetanib. Inhibiting VEGFR by vandetanib resulted in increased VEGF production in tumor tissues, paradoxically, whereas metronomic S-1 chemotherapy did not increase VEGF production. Ebos et al. [23] reported that this difference of production of VEGFs influenced to achieving malignant potential of cancer cells. Also, at this point, metronomic chemotherapy is thought to be a promising strategy of long-term treatment of cancer.

Translational/Clinical Implications of the Metronomic S-1 + Vandetanib Preclinical Results

There are several potentially important implications of our results with respect to how they might conceivably be exploited for the future treatment and management of HCC patients. It is well known that there are no effective chemotherapy regimens for the treatment of advanced HCC using conventional chemotherapy regimens. One reason for this is the frequent underlying liver dysfunction [2]. As a consequence, using MTD given in conventional schedules is often contraindicated because of possible excessive toxicity. However, chemotherapy drugs given in a metronomic, less toxic fashion may be an alternative strategy to circumvent this problem. In this regard, there is conflicting evidence regarding the clinical benefit of metronomic UFT, another 5-FU prodrug, at least in the postoperative adjuvant use for HCC [24]. However, some aspects regarding the negative clinical findings should be taken into consideration. One is the dosing. The daily dose used in the aforementioned adjuvant study was less than the dose used for a positive phase 3 adjuvant UFT clinical trial for non–small cell lung cancer patients [9]. The second is the benefit that might be gained by using an antiangiogenic drug in combination with metronomic UFT. For example, a recent report by Tang et al. showed that neither metronomic UFT nor antiangiogenic drug therapy alone had overt antitumor activity in a model of locally advanced HCC, whereas these drugs when combined showed significant antitumor activity [25]. Also, in our study, combining with vandetanib resulted in enhanced antitumor effects for S-1 chemotherapy; nevertheless, MTD S-1 monotherapy did not show any effective antitumor effects. VEGFR is related to chemoresistance for tumor endothelial cells through surviving [26]. Inhibiting VEGFR by vandetanib might have contributed to enhanced chemosensitivity for tumor endothelial cells. And EGFR is associated with resistance to 5-FU [15]. Inhibiting EGFR by vandetanib might have enhanced chemosensitivity to 5-FU. In addition, it is notable in our study that not only the combination with vandetanib but also metronomic S-1 monotherapy showed significant antitumor effects. Because S-1 may be superior to UFT in antitumor effect by virtue of its biochemical modulators [7], S-1 might be an even more suitable agent for metronomic chemotherapy.

In summary, we have demonstrated preclinically that metronomic S-1 chemotherapy showed effective therapeutic outcomes without overt toxicity for treatment of HCC, mainly by suppressing tumor angiogenesis, and the activity of which is amplified by concurrent combination with vandetanib. Metronomic S-1 and the concurrent combination treatment with an antiangiogenic agent might be a promising treatment strategy for HCC.

References

[1] Parkin D, Bray F, Ferlay J, and Pisani P (2001). Estimating the world cancer burden Globocan 2000. *Int J Cancer* **94**, 153–156.

[2] Llovet J, Di Bisceglie A, Bruix J, Kramer B, Lencioni R, Zhu A, Sherman M, Schwartz M, Lotze M, Talwalkar J, et al. (2008). Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst* **100**, 698–711.

[3] Kim JJ and Tannock IF (2005). Repopulation of cancer cells during therapy: an important cause of treatment failure. *Nat Rev Cancer* **5**(7), 516–525.

[4] Kerbel R and Kamen B (2004). The anti-angiogenic basis of metronomic chemotherapy. *Nat Rev Cancer* **4**, 423–436.

[5] Munoz R, Man S, Shaked Y, Lee C, Wong J, Francia G, and Kerbel R (2006). Highly efficacious nontoxic preclinical treatment for advanced metastatic breast cancer using combination oral UFT-cyclophosphamide metronomic chemotherapy. *Cancer Res* **66**, 3386–3391.

[6] Man S, Bocci G, Francia G, Green S, Jothy S, Hanahan D, Bohlen P, Hicklin D, Bergers G, and Kerbel R (2002). Antitumor effects in mice of low-dose (metronomic) cyclophosphamide administered continuously through the drinking water. *Cancer Res* **62**, 2731–2735.

[7] Fukushima M, Satake H, Uchida J, Shimamoto Y, Kato T, Takechi T, Okabe H, Fujioka A, Nakano K, Ohshimo H, et al. (1998). Preclinical antitumor efficacy of S-1: a new oral formulation of 5-fluorouracil on human tumor xenografts. *Int J Oncol* **13**, 693–698.

[8] Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, Miyashita K, Nishizaki T, Kobayashi O, Takiyama W, et al. (2008). S-1 plus cisplatin *versus* S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. *Lancet Oncol* **9**, 215–221.

[9] Kato H, Ichinose Y, Ohta M, Hata E, Tsubota N, Tada H, Watanabe Y, Wada H, Tsuboi M, and Hamajima N (2004). A randomized trial of adjuvant chemotherapy with uracil-tegafur for adenocarcinoma of the lung. *N Engl J Med* **350**, 1713–1721.

[10] Watanabe T, Sano M, Takashima S, Kitaya T, Tokuda Y, Yoshimoto M, Kohno N, Nakagami K, Iwata H, Shimozuma K, et al. (2009). Oral uracil and tegafur compared with classic cyclophosphamide, methotrexate, fluorouracil as postoperative chemotherapy in patients with node-negative, high-risk breast cancer: National Surgical Adjuvant Study for Breast Cancer 01 Trial. *J Clin Oncol* **27**, 1368–1374.

[11] Llovet J, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc J, de Oliveira A, Santoro A, Raoul J, Forner A, et al. (2008). Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* **359**, 378–390.

[12] Klement G, Baruchel S, Rak J, Man S, Clark K, Hicklin D, Bohlen P, and Kerbel R (2000). Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. *J Clin Invest* **105**, R15–R24.

[13] Pietras K and Hanahan D (2005). A multitargeted, metronomic, and maximum-tolerated dose "chemo-switch" regimen is antiangiogenic, producing objective responses and survival benefit in a mouse model of cancer. *J Clin Oncol* **23**, 939–952.

[14] Wedge S, Ogilvie D, Dukes M, Kendrew J, Chester R, Jackson J, Boffey S, Valentine P, Curwen J, Musgrove H, et al. (2002). ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. *Cancer Res* **62**, 4645–4655.

[15] Wang H, Jiang H, Zhou M, Xu Z, Liu S, Shi B, Yao X, Yao M, Gu J, and Li Z (2009). Epidermal growth factor receptor VIII enhances tumorigenicity and resistance to 5-fluorouracil in human hepatocellular carcinoma. *Cancer Lett* **279**, 30–38.

[16] Bocci G, Francia G, Man S, Lawler J, and Kerbel R (2003). Thrombospondin 1, a mediator of the antiangiogenic effects of low-dose metronomic chemotherapy. *Proc Natl Acad Sci USA* **100**, 12917–12922.

[17] Kamat A, Kim T, Landen CJ, Lu C, Han L, Lin Y, Merritt W, Thaker P, Gershenson D, Bischoff F, et al. (2007). Metronomic chemotherapy enhances the efficacy of antivascular therapy in ovarian cancer. *Cancer Res* **67**, 281–288.

[18] Zhang X and Lawler J (2007). Thrombospondin-based antiangiogenic therapy. *Microvasc Res* **74**, 90–99.

[19] Ghiringhelli F, Menard C, Puig P, Ladoire S, Roux S, Martin F, Solary E, Le Cesne A, Zitvogel L, and Chauffert B (2007). Metronomic cyclophosphamide regimen selectively depletes $CD4^+CD25^+$ regulatory T cells and restores T and NK effector functions in end stage cancer patients. *Cancer Immunol Immunother* **56**, 641–648.

[20] Pasquier E, Kavallaris M, and André N (2010). Metronomic chemotherapy: new rationale for new directions. *Nat Rev Clin Oncol* **7**, 455–465.

[21] Bocci G, Nicolaou K, and Kerbel R (2002). Protracted low-dose effects on human endothelial cell proliferation and survival *in vitro* reveal a selective antiangiogenic window for various chemotherapeutic drugs. *Cancer Res* **62**, 6938–6943.

[22] O'Reilly M, Boehm T, Shing Y, Fukai N, Vasios G, Lane W, Flynn E, Birkhead J, Olsen B, and Folkman J (1997). Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* **88**, 277–285.

[23] Ebos J, Lee C, Munoz W, Bjamason G, Christensen J, and Kerbel R (2009). Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. *Cell Press* **15**, 232–239.

[24] Hasegawa K, Takayama T, Ijichi M, Matsuyama Y, Imamura H, Sano K, Sugawara Y, Kokudo N, and Makuuchi M (2006). Uracil-tegafur as an adjuvant for hepatocellular carcinoma: a randomized trial. *Hepatology* **44**, 891–895.

[25] Tang T, Man S, Lee C, Xu P, and Kerbel R (2010). Impact of metronomic UFT/cyclophosphamide chemotherapy and antiangiogenic drug assessed in a new preclinical model of locally advanced orthotopic hepatocellular carcinoma. *Neoplasia* **12**, 264–274.

[26] Tran J, Master Z, Yu J, Rak J, Dumont D, and Kerbel R (2002). A role for survivin in chemoresistance of endothelial cells mediated by VEGF. *Proc Natl Acad Sci USA* **99**, 4349–4354.

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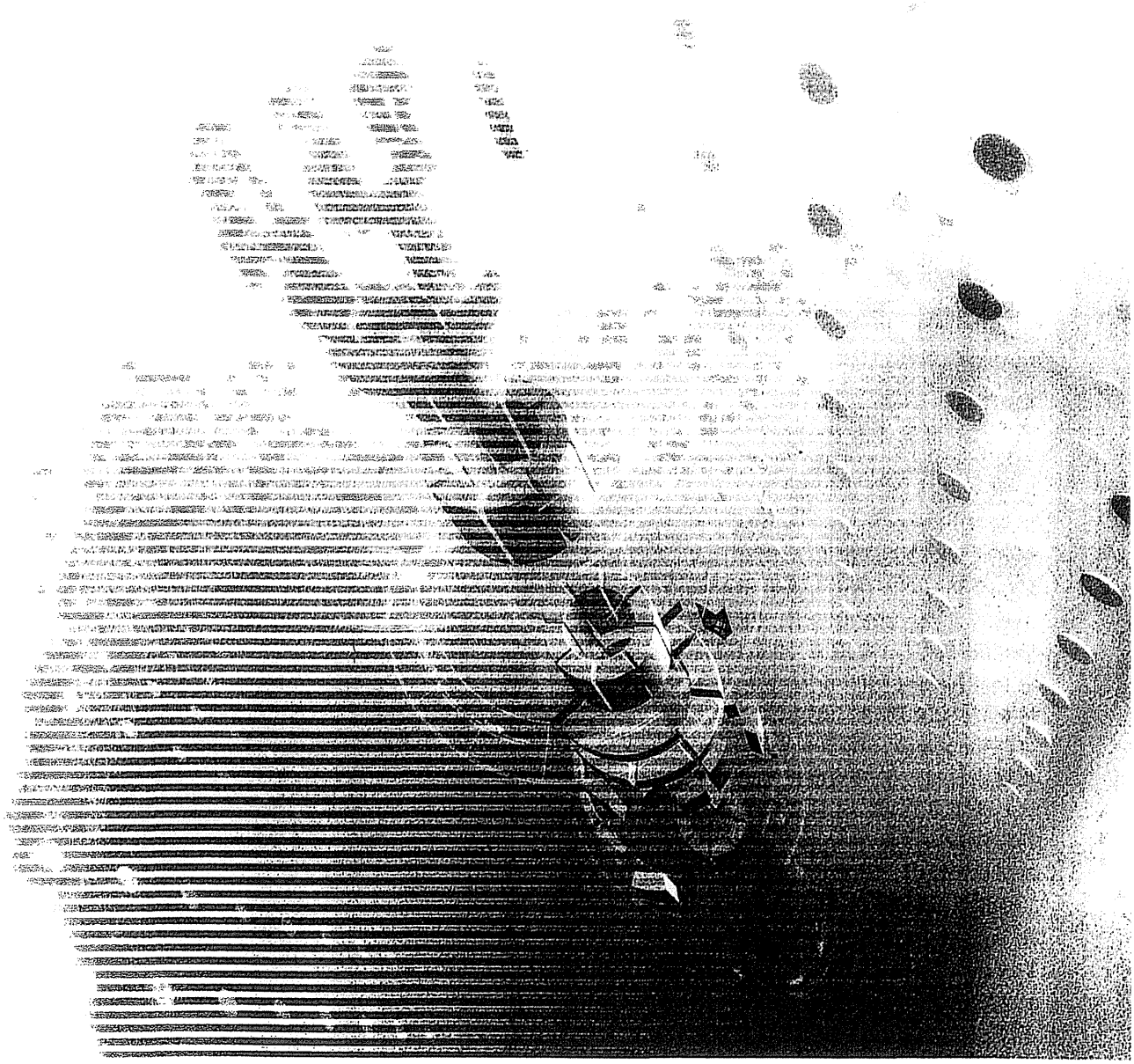

メディカルレビュー社

Session 5

ソラフェニブ投与中急速に悪化したstageⅣ-B肝細胞癌症例に対する動注化学療法

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永松 洋明[1]，岩本 英希[2]，中野 聖士[2]，鳥村 拓司[2]，佐田 通夫[2]

はじめに

進行肝細胞癌(HCC)に対する治療において，stageⅣ-Aに対する肝動注化学療法の有用性が報告されている[1)2)]。当院ではstageⅣ-Aに対してnew FP肝動注療法(NFP)[3)]を中心に行い，肝外転移がみられた時点でソラフェニブ[4)]へ変更している。しかし，ソラフェニブで効果がみられなかった場合，そのまま継続するべきか，再度ほかの治療へ変更するべきか判断に苦慮することがある。今回ソラフェニブ投与中，急速に悪化したstageⅣ-BのHCC症例に対し肝動注化学療法が有効であった3例を経験したので報告する。

症例

■症例1：64歳，男性(図1)。

病　歴：肝左葉に腫瘍径170mm，右葉に32mm，左門脈一次分枝に腫瘍塞栓を伴うstageⅣ-AのHCCに対して2005年8月からNFPを開始した。PRが得られ同年12月にはリザーバー抜去し肝左葉切除を施行した。2009年9月，腹膜播種が出現しS-1内服などsystemic chemotherapy(SC)を行うも効果はなく，2010年3月からソラフェニブの800mg/日投与へ変更した。腹膜播種は放射線治療にてコントロールできたが，6月より急速に肝内病変の悪化がみられソラフェニブを中止し，肝動注療法目的に2010年6月入院となった。

血液生化学検査：Alb 3.1g/dL，T-Bil 0.4mg/dL，PT 88.4%，WBC 5,800/μL，Hb 8.9g/dL，PLT 14.1×10⁴/μL，Child-Pugh score 6点，AFP 908ng/mL，AFP-L3 90.8%，PIVKA-Ⅱ 60,700mAU/mL。

臨床経過：肝動脈内リザーバー再留置後NFPを開始，NFPを5クール終了後肝内病変は著明に改善がみられ，PIVKA-Ⅱは6,400mAU/mLと低下した。2010年10月CT上PRが得られ，2011年2月現在生存中である。

■症例2：83歳，男性(図2)。

病　歴：肝右葉に門脈腫瘍塞栓Vp2を伴う腫瘍径122mmのHCCに対して，2008年10月からNFPを中心とした治療を開始し腫瘍縮小がみられた。2009年4月下大静脈腫瘍塞栓を伴ったHCCが再燃したが，下大静脈腫瘍塞栓に対する放射線治療(total 45Gy)と肝動注療法にて肝内病変は改善した。2009年8月に多発肺転移が出現，ソラフェニブを1日800mg/日で投与開始し，その後600mg/日へ減量した。2009年12月にはPRとなり治療継続中であったが，2010年9月肝内病変の急速な増大がみられた。

血液生化学検査：Alb 3.9g/dL，T-Bil 0.6mg/dL，PT 89.4%，WBC 6,200/μL，Hb 12.3g/dL，PLT 16.7×10⁴/μL，Child-Pugh score 5点，AFP 8,945ng/mL，AFP-L3 72.8 %，PIVKA-Ⅱ 8,945mAU/mL。

臨床経過：入院後ソラフェニブ投与を中止し，2010年9月簡易リザーバー挿入後NFPを2クール施行した。2010年11月腹部CTで肝内病変は改善し，ソラフェニブを600mg/日で再開，2011年2月現在生存中である。

■症例3：77歳，女性(図3)。

病　歴：肝S8腫瘍径32mmのHCCに対して行った2007年12月のTACE(CDDP 30mg)が初回治療であった。2008年11月と2009年8月にはHCCが多発性に再発し，TACE(EPI 30mg)を施行した。2010年7月多発肺転移，左腸骨転移がみられ，ソラフェニブを400mg/日で投与開始し1週後に600mg/日へ増量するも，副作用の

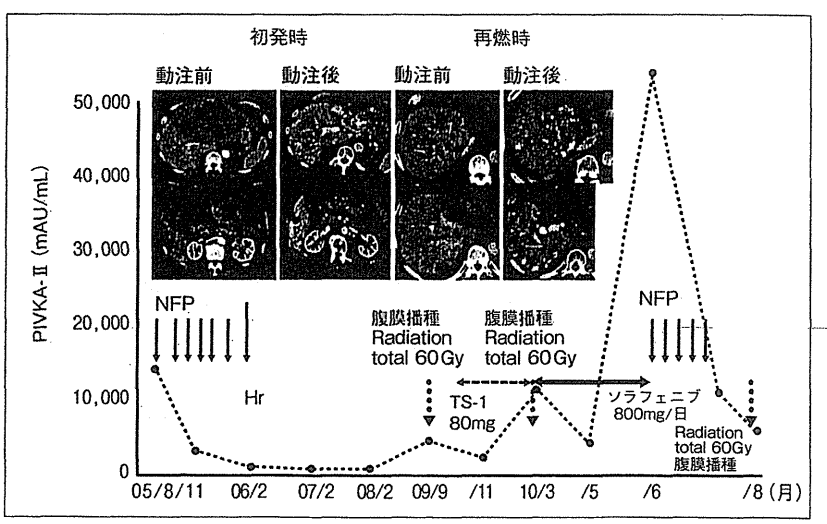

図1．【症例1】経過

ため400mg/日で維持した。骨転移へは放射線治療(total 45Gy)を併用した。同8月急速に骨転移病変が増大し疼痛増強，オピオイド鎮痛薬にて鎮痛を試みるも効果乏しく歩行困難となった。

血液生化学検査：Alb 4.2g/dL，T-Bil 1.0mg/dL，PT 92.0 %，WBC 4,800/μL，Hb 13.5g/dL，PLT 16.7×10⁴/μL，Child-Pugh score 5点，AFP 37,720ng/mL，AFP-L3 9.7%，PIVKA-Ⅱ 1,860mAU/mL。

臨床経過：内腸骨動脈へ簡易リザーバーを留置しNFPを2クール施行，疼痛の著明な改善がみられ歩行可能となり治療開始1ヵ月で退院となった。2010年11月骨盤部CTでは骨盤部腫瘍の著明な縮小がみられPRと判断された。その後2010年12月，肝内病変の再燃と肺転移の悪化がみられ，肝内へ簡易リザーバーを用いてNFPを2クール施行，PRが得られた。多発肺転移に対してはソラフェニブ投与を開始し，2011年2月現在SDの状態で生存中である。

まとめ

当院では進行HCC 22例(stageⅢ／Ⅳ-B：1／21例)へソラフェニブ投与し，病状進行や副作用のため8例は治療法を変更した。8例中5例はS-1内服[5]へ変更，3例は局所コントロールのためNFPを行い効果がみられた。ソラフェニブ治療中に病変の悪化がみられた場合，継続投与に関する有効性を示す報告は現時点ではなく，SHARP study[4]でも中止とされている。本邦の「肝がんの新規治療法に関する研究班」においては264例のソラフェニブ投与症例のうち185例が中止となっている。投与中止の理由は，病状進行が63%，副作用は22%であった。投与中止例では，後治療が行われていなかった症例が60%と半数以上であった。今回の症例検討から，状況に応じてNFPを組み合わせることは有効と考えられ，予後をさらに延長する可能性がある。この場合，動注療法NFPへ切り替えるタイミングを損なわないようにしなければならない。エビデンスがあるわけではないが，当院ではソラフェニブ投与開始1ヵ月経過の時点で画像評価し，明らかに病状が進行した場合は，症例の状況に応じて治療変更を検討している。HCCがどのような状況にあっても最善の治療を常に模索し続けることが重要である。

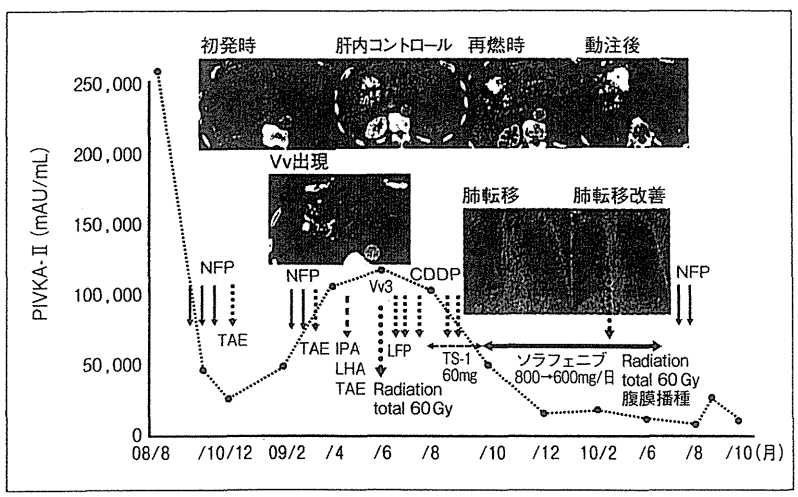

図2．【症例2】経過

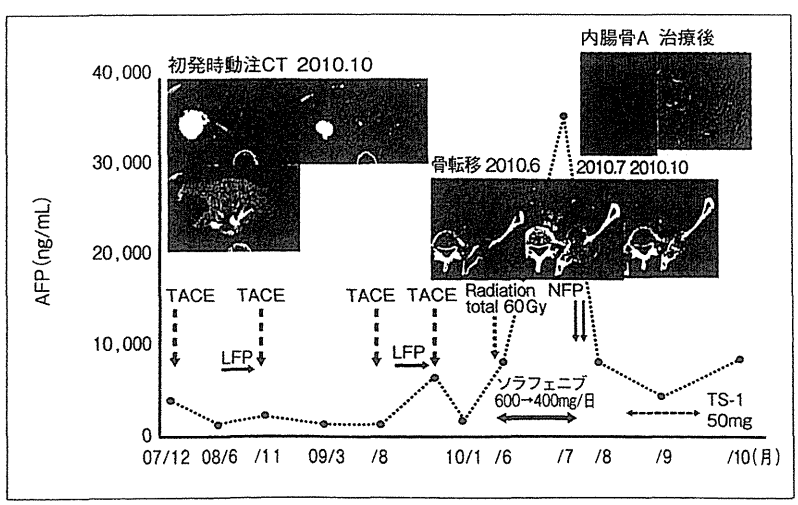

図3．【症例3】経過

References

1) Ando E, et al：Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis；analysis of 48 cases. Cancer **95**：588-595, 2002
2) Obi S, et al：Combination therapy of intraarterial 5-fluorouracil and systemic interferon-alpha for advanced hepatocellular carcinoma with portal venous invasion. Cancer **106**：1991-1997, 2006
3) Nagamatsu H, et al：Intra-arterial therapy with cisplatin suspension in lipiodol and 5-fluorouracil for hepatocellular carcinoma with portal vein thrombosis. Aliment Pharmacol Ther **32**：543-550, 2010
4) Llovet JM, et al：Sorafenib in advanced hepatocellular carcinoma. N Eng J Med **359**：378-390, 2008
5) 長田博光，他：肺転移を伴った進行肝細胞癌に対しTS-1投与を試みた8症例の検討．癌と化学療法 **34**：233-235，2007

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Original Article

Hepatitis C virus infection causes hypolipidemia regardless of hepatic damage or nutritional state: An epidemiological survey of a large Japanese cohort

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Aim: Infection with hepatitis C virus (HCV) is the leading cause of liver cirrhosis that develops into hepatocellular carcinoma. Previous studies have shown *in vitro* that lipids within hepatocytes are crucially important for a series of HCV infection–proliferation–release processes. On the other hand, in the patients with HCV, the serum total cholesterol (Total-C) and low-density lipoprotein cholesterol (LDL-C) levels have been reported to be lower. We conducted an epidemiological survey of a large cohort and investigated whether the lower serum lipid levels were caused by a direct or the secondary effects of HCV infection (i.e. hepatic damage or nutritional disorder).

Methods: Among 146 857 participants (male, 34%; female, 66%) undergoing public health examinations between 2002 and 2007 in Ibaraki Prefecture, Japan, the HCV positive rates determined by HCV antibody/antigen and/or RNA tests were 1.37% and 0.67% in males and females, respectively.

Results: In addition to Total-C and LDL-C, serum high-density lipoprotein cholesterol and triglyceride concentrations were also significantly lower in the HCV positive subjects compared with the negative subjects, regardless of sex, age or nutritional state evaluated by body mass index. Multivariate analysis showed that HCV infection was the strongest among the factors to be significantly associated with the lower level of these lipids. Particularly, the hypolipidemia was also confirmed in the HCV positive subjects with normal aminotransferase levels (alanine aminotransferase ≤30 and aspartate aminotransferase ≤30).

Conclusion: This epidemiological survey in a large Japanese cohort suggests that the HCV infection itself might directly cause hypolipidemia, irrespective of host factors including age, hepatic damage and nutritional state.

Key words: health examination, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, triglyceride

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is the leading cause of liver cirrhosis and the consequent development of hepatocellular carcinoma over time. The World Health Organization (WHO) estimates that there are approximately 180 million HCV carriers worldwide, namely, 3% of the world population, with 3–4 million new cases appearing every year, 70% of whom develop chronic hepatitis.[1,2]

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Previous studies have shown that the life cycle of HCV is strongly associated with host lipids. The HCV forms lipo-viro-particles that are transported into hepatocytes via the low-density lipoprotein (LDL) receptor.[3–6] The replication of HCV occurs where the viral replicase is assumed to localize, on the phospholipid membrane of the endoplasmic reticulum (ER) or ER-associated membrane matrix.[7] The dynamic movement of lipid droplets to the ER has been confirmed to be involved in the production of HCV particles through core protein recruitment of non-structural proteins and in some steps of virus assembly.[8] Furthermore, HCV secretion from hepatocytes is closely associated with triglyceride (TG)-rich very low-density lipoproteins.[9–11]

Several epidemiological cohort studies reported that the serum total cholesterol (Total-C) and LDL cholesterol (LDL-C) levels in HCV carriers were significantly lower than those in uninfected control subjects.[12,13] Although the reason has not been elucidated, the lower levels of serum Total-C and LDL-C were specific in HCV carriers, but not in hepatitis B virus carriers.[14–18] Recently, we have estimated that the associated parameters in the public health examination for the HCV infection based upon multivariate analysis of data from over 25 000 individuals.[19] In the result, the greatest two negatively-associated parameters for HCV carriers were serum levels of Total-C and TG, while the most positively-associated parameters were serum aminotransferase levels. Here, a question has arisen whether the hypolipidemia in the HCV carriers was caused by the impaired liver function or not, because the liver is the central organ in lipid metabolism and the decreased level of serum cholesterols has been observed in the patients with liver cirrhosis due to lower ability of cholesterol synthesis and/or malnutrition.[20,21] However, previous studies have not shown whether the hypolipidemia would occur in asymptomatic HCV carriers with normal aminotransferase levels.[22–24] Furthermore, the effects of other factors, including age, sex, nutritional state and past history of HCV infection, on serum lipid levels have not been studied in HCV carriers.

In the present study, we investigated the relations between the serum lipid profiles and the above host factors in a large cohort in public health examination with over 140 000 participants including significant numbers of asymptomatic HCV carriers without any therapies. The results showed that the hypolipidemia was a characteristic feature in HCV carriers irrespective of aminotransferase levels or nutritional states.

METHOD

Cohort study and population

THE HCV TESTING was conducted during the annual public health examination for community residents, based in part on a project for urgent comprehensive countermeasures against hepatitis and hepatocellular carcinoma at the ages of 40, 45, 50, 55, 60, 65 or 70 years, from 2002–2006, and was supported by the Japanese Ministry of Health, Labor and Welfare. Additionally, the Ibaraki Prefecture extended the project of HCV testing for an additional year to 2007, and the present study used data from a 6-year period. The present cohort study used the data from a total of 146 857 individuals (50 399 males, 34%; 96 458 females, 66%) who participated in the annual public health examinations from 2002–2007 in Ibaraki Prefecture. The HCV test was conducted with HCV antibody/antigen and/or RNA testing in accordance with the guideline for the medical HCV examination, as summarized in our previous report.[19] In the flow chart for the determination of HCV infection, using a cut-off index (COI) of the HCV antibody titer obtained with the HCV antibody test (Lumipulse; Fujirebio, Tokyo, Japan), subjects were initially divided into the HCV negative with COI of less than 1, the HCV positive candidates with COI of $1 \leq \text{COI} < 50$ and the HCV positive with COI of 50 or more. The HCV positive candidates were finally determined to be HCV negative and positive based upon the HCV antigen test for the HCV core protein and the nucleic acid amplification test (NAT) for HCV RNA.

The health examination involved measurements of serum lipid levels, including Total-C, high-density lipoprotein cholesterol (HDL-C) and TG, as well as age, height, weight and serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). According to the general health examination, serum was collected on fasting. Serum LDL-C levels were calculated using the Friedewald formula, as follows: LDL-C (mg/dL) = Total-C (mg/dL) – HDL-C (mg/dL) – 0.2 × TG (mg/dL).[25] Over 802 mg/dL (8.8 mmol/L) of TG level was excluded from the calculation of LDL-C.[26] The lipid levels were diagnosed as indicating normal, hypolipidemia or hyperlipidemia based on the respective reference value for Japanese clinical laboratory examination.[27,28] Body mass index (BMI) was calculated by dividing the weight (Wt) in kilograms by the square of the height in meters.[29] All of the health examinations, including HCV tests and serum biochemical analyses, were conducted in the Ibaraki Health Service Association and Ibaraki Prefectural Institute of Public Health (Mito, Japan), and the data of health examination were analyzed anonymously, after informed consent was obtained from community representatives to conduct an epidemiological study based on the guidelines of the Council for International Organizations of Medical Science.[30]

Classification by factors

In the present study, both HCV negative and positive subjects were further divided into subgroups based upon different factors: (i) sex; (ii) age; (iii) serum HCV

antibody titer; (iv) serum markers of liver damage; and (v) nutritional state. The age classification was established by the age range, and was divided into 5-year increments. In the classification by serum HCV antibody titer, the HCV negative subjects were divided into two subgroups, HCV antibody titer COI of less than 1 and COI of 1 or more, and the subjects with COI of 1 or more were finally decided as being HCV negative by the HCV antigen test and NAT.[19] For classification by liver damage, the HCV negative and positive subjects were further divided into the two groups, based upon the healthy limits of serum aminotransferases (ALT and AST): "normal" was less than 30 IU of both, and "abnormal" was over 30 IU of either or both aminotransferases. In Japan, the healthy limits of both serum aminotransferase levels for diagnosis of liver damage in public health examinations were re-established to be under 30 IU, based on the recent guideline for antivirus therapy for HCV.[31] The nutritional status was evaluated by BMI, and the classification was conducted along with the WHO-defined BMI class: under Wt was BMI of less than 18.5, normal Wt of $18.5 \leq \text{BMI} < 25$, over Wt of $25 \leq \text{BMI} < 30$ and obese class according to obese classes 1–3 (BMI >30).

Statistical analysis

Data are expressed as the mean ± standard error of the value or percentage. Significant differences between the two groups were determined by unpaired Student's *t*-test or Mann–Whitney *U*-test depending upon the number of subjects and variations in the groups compared. Comparison of the percent distribution between the two groups was estimated by Pearson's χ^2-test analysis. Multivariate logistic regression analysis was performed to determine factors including HCV positive, age, BMI, ALT and AST associated with serum level of each lipid diagnosed as the hypolipidemia (Total-C ≤119 mg/dL, HDL-C ≤39 mg/dL in males and ≤44 mg/dL in female, LDL-C ≤64 mg/dL, TG ≤49 mg/dL). The strength of association was described with an odds ratio with 95% confidence intervals and *P*-value. The statistical analysis was performed using SPSS II software version 11.0.

RESULT

HCV positive rate and profile of serum lipids between HCV positive and negative

AMONG THE 146 857 individuals who participated in the health examination from 2002–2007, the HCV positive rates were 0.90%, 1.37% and 0.67% in all (sum of the sexes), males and females, respectively. There were no significant differences in BMI between the HCV negative (male, 23.9 ± 0.01; female, 23.1 ± 0.01) and positive (male, 23.3 ± 0.1; female, 23.1 ± 0.1) subjects. Table 1 shows the average serum lipid levels (Total-C, HDL-C, LDL-C and TG) by sex between the HCV positive and negative subjects. Among all subjects, all serum lipids in the HCV positive subjects were significantly lower than in the HCV negative subjects, regardless of sex.

The lipid levels in both HCV negative and positive subjects were divided into hypolipidemia, normal lipid and hyperlipidemia, based upon whether they were below, within and above the normal ranges of the respective reference values for Japanese (Fig. 1). Among both sexes, the proportion that were above the normal range for all examined lipids was significantly lower in the HCV positive compared to those in the HCV negative subjects (χ^2-test analysis $P < 0.0001$ in all: Total-C, 29% in the HCV negative vs 6% in the HCV positive for males, 41% vs 21% in females; HDL-C, 3% vs 1% in males, 6% vs 4% in females; LDL-C, 24% vs 7% in males, 34% vs 20% in females; TG, 35% vs 18% in males, 21% vs 14% in females).

The HCV negative subjects were also divided into those with HCV antibody titer of 1 or more and less than 1, and the former and latter were considered as having a prior infection and never infected.[17] The percentages of HCV negative subjects with prior infection were 0.91%, 1.28% and 0.72% for all, males and females, respectively, and the number of subjects was similar to the HCV positive subjects for each sex. Significant differences in the serum lipids were observed when the HCV positive subjects were compared regarding the presence or absence of a prior infection (Table 1). Among the HCV negative subjects, the examined lipids tended to be lower in those with prior infection compared with those who had never been infected, particularly in males, but there were no statistically significant differences.

Table 2 shows the multivariate logistic regression analysis of risk factors for lower level of serum lipids. In the parameters including HCV positive, age, ALT, AST and BMI, the significances were recognized in almost all analyses for the respective lower level of serum lipids in both sexes, while there were no significances in age for Total-C in male, ALT and BMI for Total-C in female, and both aminotransferases for LDL-C in female. In the HCV positive parameter of both sexes, the odds ratios in all examined lipids were remarkably higher than other analyzed

Table 1 Profile of serum lipids between the HCV negative and positive subjects by sex

		Total-C (mg/dL)		HDL-C (mg/dL)		LDL-C (mg/dL)		TG (mg/dL)	
All									
HCV positive	(n = 1317)	179.2 ± 1.0		52.9 ± 0.4		105.2 ± 0.3		107.6 ± 1.9	
HCV negative	(n = 145 540)	209.3 ± 0.1	**	60.3 ± 0.04	**	124.3 ± 0.1	**	124.5 ± 0.2	**
Titer ≥1	(n = 1326)	204.2 ± 1.0	**	57.3 ± 0.4	**	121.6 ± 0.9	**	127.5 ± 2.2	**
Titer <1	(n = 144 214)	209.4 ± 0.1	**	60.3 ± 0.04	**	124.3 ± 0.1	**	124.5 ± 0.2	**
Male									
HCV positive	(n = 679)	168.1 ± 1.2		48.3 ± 0.5		98.0 ± 1.1		112.0 ± 3.1	
HCV negative	(n = 49 720)	202.1 ± 0.2	**	54.9 ± 0.1	**	118.3 ± 0.1	**	155.7 ± 0.5	**
Titer ≥1	(n = 638)	195.4 ± 1.3	**	53.3 ± 0.6	**	114.7 ± 1.3	**	139.3 ± 3.5	**
Titer <1	(n = 49 082)	202.3 ± 0.2	**	54.9 ± 0.1	**	118.4 ± 0.1	**	155.9 ± 0.5	**
Female			(vs male)		(vs male)		(vs male)		(vs male)
HCV positive	(n = 638)	191.0 ± 1.4	(**)	57.8 ± 0.6	(**)	112.8 ± 1.2	(**)	102.9 ± 2.3	(*)
HCV negative	(n = 95 820)	213.0 ± 0.1	** (**)	63.1 ± 0.1	** (**)	127.3 ± 0.1	** (**)	113.3 ± 0.2	** (**)
Titer ≥1	(n = 688)	212.4 ± 1.3	** (**)	61.0 ± 0.5	** (**)	128.1 ± 1.2	** (**)	116.5 ± 2.5	** (*)
Titer <1	(n = 95 132)	213.0 ± 0.1	** (**)	63.1 ± 0.1	** (**)	127.3 ± 0.1	** (**)	113.2 ± 0.2	** (**)

The titer ≥1 and <1 show the HCV negative subjects with HCV antibody titer over 1 and more, and less than 1, respectively. Data are shown the mean ± standard error. Significant differences between the HCV positive and HCV negative subjects and between sexes were analyzed by Mann–Whitney U-test. *P < 0.05, **P < 0.0001. Symbols in the parenthesis in female show the significant difference compared to that in male. LDL-C value was calculated using the Friedewald formula (LDL-C = Total-C – HDL-C – TG / 5).
HCV, hepatitis virus C; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; Total-C, total cholesterol.

Table 2 Multivariate logistic regression analysis of factors associated with hypolipidemia

	Total-C			HDL-C			LDL-C			TG		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Male												
HCV (+)	10.75	7.00–16.50	<0.0001	3.09	2.55–3.74	<0.0001	2.10	1.62–2.72	<0.0001	3.17	2.37–4.26	<0.0001
Age (years)	1.01	0.99–1.03	0.1180	1.02	1.01–1.02	<0.0001	0.98	0.99–0.99	<0.0001	0.99	0.98–0.99	<0.0001
ALT	1.01	1.01–1.02	<0.0001	0.98	0.98–0.99	<0.0001	1.04	1.04–1.05	<0.0001	1.02	1.02–1.02	<0.0001
AST	1.00	0.99–1.00	0.1870	1.02	1.01–1.02	<0.0001	0.98	0.98–0.99	<0.0001	0.96	0.96–0.97	<0.0001
BMI	0.89	0.85–0.94	<0.0001	1.15	1.14–1.16	<0.0001	0.95	0.94–0.97	<0.0001	0.80	0.78–0.81	<0.0001
Female												
HCV (+)	14.93	6.90–32.27	<0.0001	2.24	1.81–2.78	<0.0001	3.67	2.40–5.63	<0.0001	1.38	1.00–1.99	0.0479
Age (years)	0.94	0.92–0.96	<0.0001	1.03	1.03–1.03	<0.0001	0.93	0.93–0.94	<0.0001	0.94	0.94–0.94	<0.0001
ALT	1.02	1.00–1.04	0.1034	0.98	0.98–0.99	<0.0001	1.01	1.00–1.02	0.0566	1.03	1.03–1.04	<0.0001
AST	0.99	0.97–1.01	0.4201	1.02	1.01–1.02	<0.0001	1.00	1.00–1.01	0.5252	0.96	0.96–0.97	<0.0001
BMI	0.88	0.82–0.94	0.0002	1.13	1.12–1.14	<0.0001	0.96	0.94–0.97	<0.0001	0.84	0.84–0.85	<0.0001

The lower level of each serum lipid was defined as below the normal range of the respective reference value for Japanese, and see Figure 1 for the values.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; HCV (+), positive for hepatitis C virus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; TG, triglyceride; Total-C, total cholesterol.

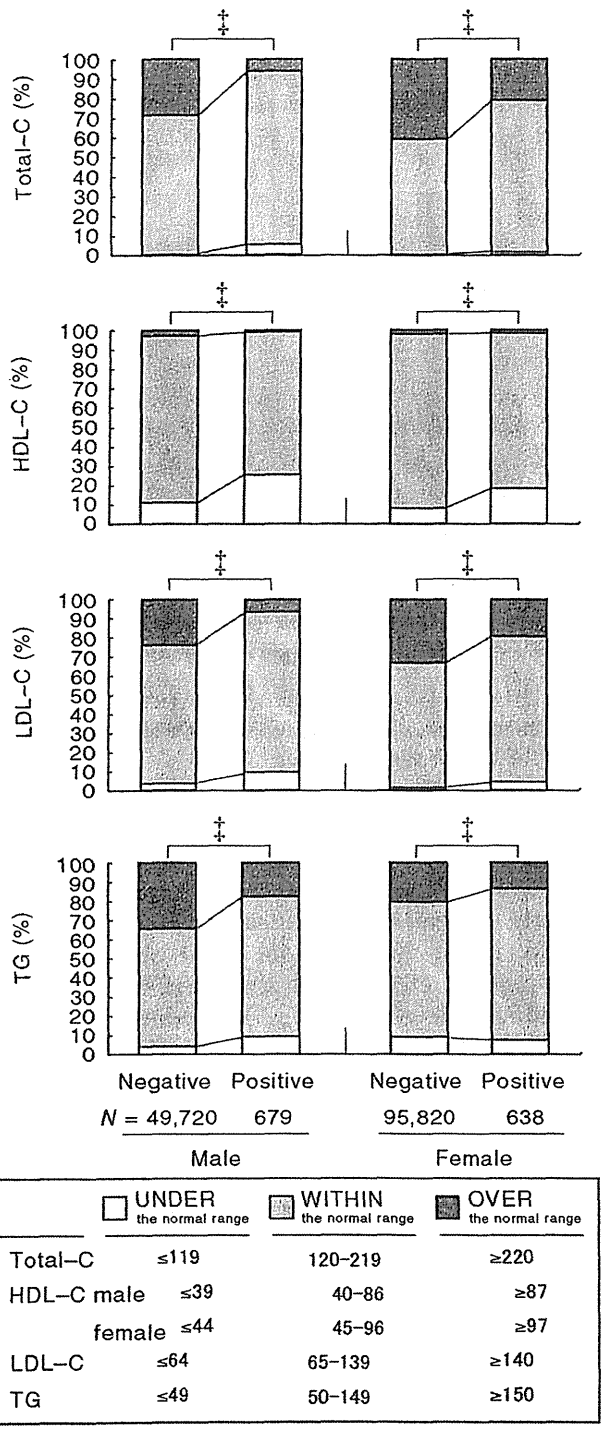

Figure 1 Comparison of the relative ratios of the three classifications of lipids based on the reference values for Japanese clinical examination, between the hepatitis C virus (HCV) negative and positive patients. The respective lipids were divided into under, within and over normal ranges. ‡$P < 0.001$ shows a significant difference of the relative ratio between the HCV negative and positive subjects by Pearson's χ^2-test analysis. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; Total-C, total cholesterol.

parameters in all examined lipids. Although this analysis implied that the influence of HCV infection was the strongest risk factor for the lower level of serum lipids, the further analyses by matching sex, age, ALT, AST and BMI were carried out to exclude these factors.

Composition of serum lipids between the HCV positive and negative subjects

Figure 2 shows the balance of serum lipid composition by sex between HCV positive and negative subjects. In both sexes, there was no significant differences in the balance of serum lipid composition between HCV positive and negative subjects. Among males, the rates of TG and HDL-C in the HCV positive subjects tended to be lower and higher, respectively, compared with the HCV negative subjects (TG, $42.4 \pm 0.1\%$ vs $40.5 \pm 0.5\%$; HDL-C, $18.7 \pm 0.03\%$ vs $20.2 \pm 0.3\%$, in the HCV negative vs positive subjects, respectively), but they were not statistically significant. The serum lipid balance in females was almost the same between the HCV negative and positive subjects. The results show that the all serum lipids were reduced equally in subjects with HCV infection.

Serum levels of lipids classified by healthy levels of aminotransferases

The HCV negative and positive subjects were classified into the normal (ALT ≤30 and AST ≤30) and abnormal (ALT >30 and/or AST >30) populations based upon the healthy serum aminotransferase levels. The HCV positive rates were 0.36% and 3.30% in the normal and abnormal populations, respectively. In the HCV negative subjects, 82.1% were in the normal compared with 17.9% that were in the abnormal population (χ^2-test analysis $P < 0.0001$). In contrast, the normal and abnormal populations in the HCV positive were 33.1% and 66.9% ($P < 0.0001$), respectively. Serum lipid levels classified by the aminotransferases are shown in Figure 3. There were significant differences in the lipid levels between the HCV negative and positive subjects in the normal population. In both sexes, all examined lipid levels in the

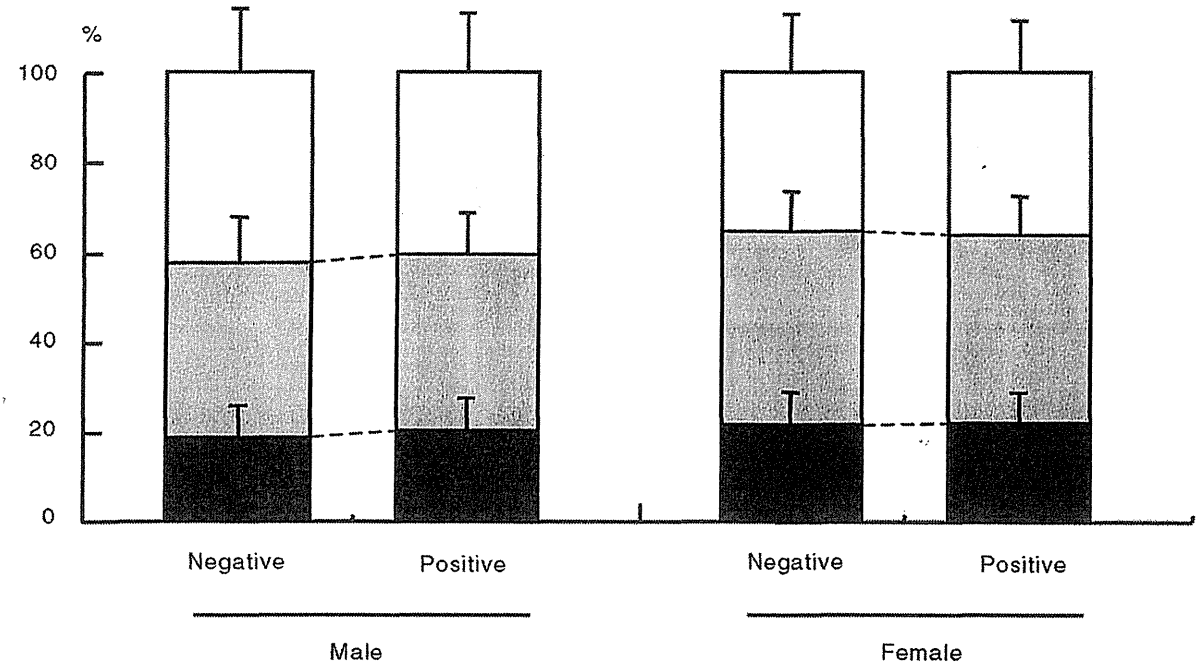

Figure 2 Composition of serum high-density lipoprotein cholesterol (■ HDL-C), low-density lipoprotein cholesterol (▨ LDL-C) and triglyceride (□ TG) for each sex between the hepatitis C virus positive and negative subjects. Data are shown as mean ± standard deviation of the respective percentage in TG, LDL-C and HDL-C for sum of them.

normal population were significantly lower in the HCV positive compared with the negative subjects, except for TG in females. The significantly lower levels of all examined lipids in the HCV positive were also observed in the abnormal population. The results indicate that the lower levels of serum lipids were associated with the infection of HCV rather than the condition of liver damage.

Differences of lipids among age ranges

Figure 4 shows the differences in the age range of serum lipid levels in 5-year increments in the HCV negative and positive subjects by sex. In both sexes, lower levels of all examined lipids in the HCV positive subjects were observed for all age ranges, except for some younger age ranges for TG level. Among the age ranges under 50 years, the Total-C and LDL-C levels in the HCV negative subjects were lower in females than in males, but the lower levels were reversed in those aged above 50 years. In the HCV positive subjects, however, both levels were weakly influenced by age for both sexes, and therefore the lower levels in males remained unchanged throughout all age ranges.

Serum levels of lipids classified by BMI

Figure 5 shows the serum lipid levels classified by the WHO-defined classification of BMI. In all BMI classes for both sexes, except for the under Wt class in females, Total-C and LDL-C levels in the HCV positive subjects were significantly lower than in the negative subjects. Similarly, a significant decrease of HDL-C levels was observed in the BMI classes for both sexes in the HCV positive group, except for in the obese class who also showed lower levels; however, this finding was not significant. In TG, lower levels were observed in the HCV positive subjects for all BMI classes in both sexes, and significant differences were found in the normal Wt and over Wt classes for both sexes and for the obese class in males. Accompanied with the higher class of BMI, the typical dyslipidemic patterns of higher TG and lower HDL-C levels were observed in both HCV positive and negative subjects, but the effects of BMI were smaller in HCV positive than in negative subjects.

DISCUSSION

AMONG OVER 140 000 participants undergoing public health examinations, we evaluated the serum lipid profiles in the HCV positive subjects by various host factors including sex, age, nutritional state, hepatic damage and HCV antibody titer. In contrast to HCV hepatitis patients in hospitals, this cohort included