Original Contribution

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Dosage Escalation Study of S-1 and Irinotecan in Metronomic Chemotherapy against Advanced Colorectal Cancer

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Summary: The anti-angiogenic efficacy of chemotherapy would seem to be optimized by administering comparatively lower doses of drugs on a more frequent (daily, several times a week, or weekly) or continuous schedule, with no extended interruptions – sometimes referred to as 'metronomic' chemotherapy. This phase I study was performed to determine the recommended dosage (RD) of metronomic chemotherapy using oral fluoropyrimidine S-1 plus weekly irinotecan (CPT-11) in patients with previously untreated advanced or recurrent colorectal cancer. Patients received first-line chemotherapy consisting of 80 mg/m^2 of S-1 given on days 3 to 7, 10 to 14, and 17 to 21 with escalating dosages of CPT-11 (from 40 mg/m^2) administered intravenously on day 1, 8, and 15 of a 28-day cycle. Standard patient eligibility criteria were used. Based on the concept of metronomic chemotherapy, dose limiting toxicity (DLT) was defined any toxicity that resulted in skipping of CPT-11 administration, or more than 5 days suspension in S-1 administration, in addition to the conventional criteria. If the maximum tolerated dosage (MTD) was defined as the maximum dosage at which no suspension of CPT-11 or S-1 administration occurred, the RD was considered to be the dosage one rank lower than the MTD. On the other hand, in the present study the MTD was defined as the dosage at which at least one suspension of CPT-11 or S-1 administration occurred, the MTD was considered to be the RD. Two of the first 3 patients at level 4 received 60 mg/m^2 of CPT-11 and 80 mg/m^2 of S-1 experienced a suspension in CPT-11 administration, thus level 4 was defined as the MTD and RD. Sixty mg/m^2 of CPT-11 and 80 mg/m^2 of S-1 were the indicated RD for the following phase Ⅱ study of metronomic chemotherapy.

Key words metronomic chemotherapy, S-1, irinotecan, advanced colorectal carcinoma

INTRODUCTION

Irinotecan (CPT-11) is a key drug in the management of metastatic colorectal cancer, as demonstrated by several randomized studies indicating a survival benefit. It has been shown that the response rate to CPT-11 was 11 to 25% in patients with advanced colorectal cancer refractory to 5-fluorouracil (5-FU) based chemotherapy [1,2]. These findings implied a lack in tumor cross-resistance to the two agents CPT-11 and 5-FU. Moreover, favorable results from combination chemotherapy using CPT-11 and 5-FU/leucovorin (LV) for advanced colorectal cancer have been reported [3,4], and a CPT-11 and infusion plus bolus

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Abbreviations: BSA, body surface area; CEA, carcinoembryonic antigen; CDHP, 5-chloro-2,4-dihydrooxypyridine; CPT-11, irinotecan; CR, complete response; CT, computed tomography; DLT, dosage limiting toxicity; ECG, electrocardiogram; ECOG, Eastern Cooperative Oncology Group; 5-FU, 5-fluorouracil; FBAL, F-beta-alanine; FOLFIRI, folnic acid+5-FU+irinotecan; FOLFOX, folnic acid+5-FU+oxaliplatin; LV, leucovorin; MTD, maximum tolerated dosage; NCI-CTC, National Cancer Institute Common Toxicity Criteria; Oxo, potassium oxonate; PD, disease progression; PR, partial response; PS, performance status; RECIST, Response Evaluation Criteria in Solid Tumors; RD, recommended dosage; SD, stable disease; UFT, uracil plus tegafur; VEGF, vascular endothelial growth factor.

5-FU/LV regimen FOLFIRI (folnic acid+5-FU+irinotecan) has been recommended as first-line therapy for advanced colorectal cancer, as well as FOLFOX (folnic acid+5-FU+oxaliplatin) regimens which added oxaliplatin to intravenous 5-FU/LV [5]. Since these regimens have consisted of the conventional maximum tolerated dosages (MTDs) of CPT-11 and 5-FU, adverse effects of grade 3 or worse are not uncommon. Moreover, administration of infusion 5-FU is becoming more complex because of the need for vascular access devices and a portable delivery system.

S-1 is an oral fluoropyrimidine preparation developed by Taiho Pharmaceutical Co., Ltd. (Tokyo, Japan) that combines tegafur with two 5-FU modulators 5-chloro-2,4-dihydrooxypyridine (CDHP) and potassium oxonate (Oxo) at a molar ratio of 1:0.4:1 [6]. Tegafur, a prodrug of 5-FU, is converted to 5-FU mainly in the liver and in the tumor cells. CDHP, a reversible inhibitor of dihydropyrimidine dehydrogenase, suppresses the degradation of 5-FU, thereby maintaining high concentrations of 5-FU in plasma and the tumor cells [6,7]. CDHP also decreases the cardiotoxic and neurotoxic effects by reducing the production of F-beta-alanine (FBAL), the main catabolite of 5-FU [8,9]. After peroral administration, Oxo is selectively distributed to the small and large bowels. High concentrations of Oxo in these organs inhibit the phosphorylation of 5-FU to fluoropyrimidine monophosphate, catabolized by orotate phosphoribosyltransferase within the gastrointestinal mucosal cells, thereby reducing the incidence of diarrhea [10].

In phase Ⅱ trials of S-1 as a single agent, response rates ranging from 19 to 39% were obtained in patients with advanced colorectal cancer [11-13]. These studies demonstrated that S-1 had a high response rate and good compliance in patients with advanced colorectal cancer treated on an outpatient basis. Several regimens combining S-1 and CPT-11 were subsequently developed. Goto et al. [14] conducted a phase Ⅱ study consisting of 150 mg/m^2 of CPT-11 given on day 1 with 40 mg/m^2 of S-1 twice daily on days 1 to 14 of a 21-day cycle to assess efficacy and safety. They concluded that the combined treatment with S-1 and CPT-11 was a promising regimen, offering benefits in terms of safety and survival as compared with conventional regimens such as FOLFIRI in patients with advanced colorectal cancer.

Conventional cytotoxic chemotherapeutics affect the endothelium of the growing tumor vasculature in addition to affecting the proliferating cancer cells and various types of normal cells [15]. The antiangiogenic efficacy of chemotherapy would seem to be optimized by administering comparatively low dosages of drugs on a more frequent (daily, several times a week, or weekly) or continuous schedule, with no extended interruptions – sometimes referred to as 'metronomic' chemotherapy [16]. This would also have the advantage of being less acutely toxic, therefore making more prolonged treatments hypothetically possible. Thus, peroral fluoropyrimidine on a daily schedule would be a typical metronomic chemotherapy. In fact, the antitumor efficacy of capecitabine or UFT (uracil plus tegafur)/LV is not inferior to that of intravenous 5-FU/LV, and has lower toxicity in advanced colorectal cancer [17,18]. However, since grade 3 or 4 toxicities can be expected to necessitate temporary suspension of the chemotherapy, a high dosage of bi-weekly or tri-weekly CPT-11 as in Goto's regimen [14] might not realize the metronomic advantage of daily peroral fluoropyrimidine in combination therapy of CPT-11 and peroral fluoropyrimidine. We have previously reported the safety and efficacy of metronomic chemotherapy using low-dosage weekly CPT-11 and daily 5'-deoxy-5-fluorouridine, an intermediate metabolite of capecitabine, for advanced colorectal cancer, even in elderly patients [19]. Thus, combined metronomic administration of CPT-11 and peroral fluoropyrimidine is expected to be useful even in poor risk patients who are unable to receive standard CPT-11 and 5-FU (or S-1) chemotherapy. We have therefore postulated that a combination therapy of weekly CPT-11 and S-1 could realize the advantages of metronomic chemotherapy by having an antiangiogenic effect in addition to an antiproliferation effect. We conducted this phase I study to assess the recommended dosage (RD) of weekly CPT-11 combined with daily S-1 as a metronomic chemotherapy, in which treatment can be continued without temporary interruption.

PATIENTS AND METHODS

Eligibility

This was a non-randomized, open-label, phase I, dosage-escalating study, performed at Kurume University Hospital, between April 2004 and December 2005. The criteria for eligibility were histological findings of colorectal carcinoma indicating nonresectable metastatic or recurrent disease, no prior chemotherapy, no major surgery or radiation therapy within 2 weeks of beginning treatment, measurable tumors with at least one lesion having dimensions $\geqq$10 mm in longest diameter, a life expectancy of $\geqq$3 months and a

performance status (PS) according to the Eastern Cooperative Oncology Group (ECOG) criteria of 0-1, adequate bone marrow function (leucocytes ≧4000 per mm^3, platelets ≧100000 per mm^3), adequate liver function (bilirubin ≦1.5 mg/dl), adequate renal function (creatinine ≦1.1 mg/dl), no serious or uncontrolled concurrent medical illness, and no active other malignancy. Postoperative adjuvant chemotherapy excluding regimens including CPT-11 or S-1 was allowed. Patients were required to be ≧20 years and <75 years of age, and non-pregnant. All patients were informed of the investigative nature of this treatment, and gave their fully-informed written consent. This study was approved by the ethics committee of Kurume University (No. 2355).

Treatment protocol

CPT-11 was administered by infusion intravenously over 90 min for three consecutive weeks followed by one week of rest, in 4-week treatment cycles. S-1 was available as capsules containing 20 or 25 mg of tegafur. S-1 was given perorally twice daily on days 3 to 7, 10 to 14, and 17 to 21. Patients were assigned one of the following dosages, to be taken within an hour after breakfast and supper, on the basis of body surface area (BSA); level 1: 25 mg (BSA<1.25 m^2), 40 mg (BSA≧1.25 to <1.50 m^2), or 50 mg (BSA≧1.50), level 2 or after: 40 mg (BSA<1.25 m^2), 50 mg (BSA≧1.25 to <1.50 m^2), or 60 mg (BSA≧1.50). Cycles were repeated every 4 weeks (Fig. 1).

The CPT-11 administration was temporarily suspended for grade 2 or higher mucositis, any grade of diarrhea, grade 3 or higher other non-hematological toxicity, or for leucocytes <3000/mm^3, granulocytes <1500/mm^3, or platelets <100000/mm^3. The S-1 administration was also temporarily suspended for grade 2 or higher diarrhea, grade 2 or higher mucositis, grade 3 or higher other non-hematological toxicity, or for leucocytes <2000/mm^3, granulocytes <1000/mm^3, or platelets <75000/mm^3. The therapy was alternatively re-instituted using a dosage of minus 1 level after recovery from all toxicities, if leucocytes <2000/mm^3, granulocytes <1000/mm^3, platelets <50000/mm^3, or grade 3 or higher non-hematological toxicity (excluding nausea/vomiting and general fatigue) was noted during the cycle, or in any case where the treatment delay was longer than 14 days before the start of the next cycle of treatment. All treatment was performed on an outpatient basis.

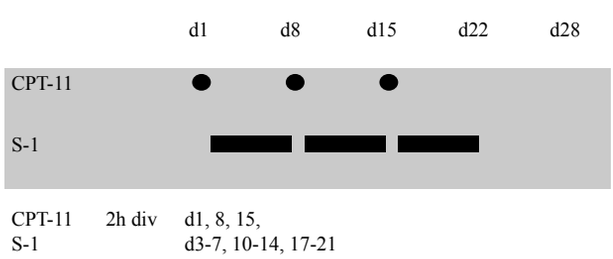

Fig. 1. Scheme of treatment protocol.

Dosage escalation

The dosage of S-1 was fixed at 80 mg/m^2 after level 2. The dosage of CPT-11 was started at 40 mg/m^2 and escalated after level 2, as shown in Table 1. Up to 6 patients at each dosage level completed one cycle of treatment before the next dosage level was tried. The dosage at which ≧2 of 3 or ≧2 of 6 patients experienced dosage limiting toxicity (DLT) during the first cycle of treatment was defined as the MTD. Adverse reactions were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 3.0. Toxicity and laboratory variables were assessed weekly during the first and second treatment cycle. DLT was defined during the first treatment cycle as: grade 4 neutropenia, grade 4 thrombocytopenia, grade 3 neutropenia associated with fever ≧38°C, grade 3 or more non-hematological toxicity with the exception of alopecia, nausea and vomiting, and treatment delay of >7 days before the second cycle of treatment. Any skip in CPT-11 administration or more than 5 days suspension of S-1 administration with no relationship to the above DLTs was defined as an additional DLT.

Assessment of recommended dosage (RD)

If MTD is defined as the maximum dosage at which there was no skipping of CPT-11 or more than 5 days suspension in S-1 administration as an additional DLT, the RD is generally considered to be the dosage one rank lower than the MTD. However, for

TABLE 1.
Dose escalation scheme

	S-1	CPT-11
Level 1	60 mg/m^2	40 mg/m^2
Level 2	80 mg/m^2	40 mg/m^2
Level 3	80 mg/m^2	50 mg/m^2
Level 4	80 mg/m^2	60 mg/m^2

S-1 80 mg/m^2/day
BSA<1.25 80 mg/body/day
1.25≦BSA<1.5 100 mg/body/day
1.5≦BSA 120 mg/body/day
CPT-11: irinotecan, BSA: body surface area.

the purposes of the present study if MTD is defined as the dosage at which at least one additional DLT of CPT-11 skipping or more than 5 days suspension in S-1 administration occurs, the RD is considered to be the same as the MTD at which a majority of patients will tolerate conventionally.

Follow-up evaluation

Within 2 weeks before initiating the chemotherapy, all patients were assessed by a physical examination, routine hematology and biochemistry analyses, ECG (electrocardiogram), chest X-ray and abdominal computed tomography (CT) scans to define the extent of disease. Complete blood cell counts with platelet and differential counts were recorded weekly during chemotherapy, and serum chemistries were repeated once or twice within every treatment cycle. Subjective symptoms, body weight, physical examination, performance status, and all adverse effects were recorded before each treatment course. Measurement of serum tumor marker carcinoembryonic antigen (CEA) level was performed at least once every 4 weeks.

Assessment of the objective response rate

Measurable lesions were reassessed every 8 weeks using CT scan, X-ray, and other techniques that allowed retrospective and independent evaluation. The response rate was assessed every 8 weeks using the RECIST (Response Evaluation Criteria in Solid Tumors) criteria. In cases of partial response or complete response, a further assessment at 4 weeks later was required for confirmation of the response; all tumor measurements were reviewed and confirmed by an independent panel of radiologists.

RESULTS

Patient characteristics

A total of 16 patients entered this study. The patient characteristics are shown in Table 2. The average age was 60 years, ranging from 36 to 74 years. Seven were male, and 9 were female. The PS according to the ECOG criteria was 0 in the majority of patients. Seven patients presented one affected organ, 8 patients presented two, and 1 patient presented three affected organs, with the most commonly affected organ being the distant lymph nodes. All eligible patients received at least 2 cycles of treatment.

MTD and RD

Adverse effects and DLT are shown in Table 3. Patient no. 2 experienced grade 3 diarrhea as a DLT after the third administration of CPT-11 on day15 at level 1.

TABLE 2.
Patient's characteristics

	Pt No.	Age	Sex	PS	Primary site	Affected organs	Treatment course
Level 1	1	53	M	0	Colon	Mediastinal LN, Spine	7
	2	63	M	1	Colon	Peritoneum	2
	3	36	F	0	Colon	Paraaortic LN	4
	4	55	M	1	Rectum	Intrapelvic, Peritoneum	4
	5	56	F	0	Colon	Mediastinal LN	4
	6	60	F	1	Colon	Liver, Lung, Neck LN	7
Level 2	7	74	M	0	Rectum	Lung	4
	8	57	F	0	Coon	Lung	5
	9	74	M	0	Rectum	Liver, Lung	4
Level 3	10	67	M	1	Colon	Mediastinal LN	2
	11	56	F	0	Colon	Iliac LN	8
	12	63	F	0	Colon	Peritoneum, Paraaortic LN	8
Level 4	13	53	F	1	Colon	Liver, Lung, Paraaortic LN	6
	14	57	F	0	Colon	Liver, Peritoneum	7
	15	58	M	0	Rectum	Lung, Paraaortic and iliac LN	2
	16	60	F	0	Rectum	Liver, Lung, Peritoneum, Primary	3

PS: performance status according to the Eastern Cooperative Oncology Group criteria.
M: male, F: female, LN: lymph nodes

TABLE 2.
Adverse effects during the first cycle, DLT and tumor response

	Pt No.	Adverse effects	DLT	Tumor Response
Level1	1	Fatigue (2)		SD
	2	Nausea (1), Fatigue (1)	Diarrhea (3)	SD
	3	Nausea (2), Fatigue (1)		SD
	4			PD
	5	Nausea (2), Fatigue (2)		PR
	6	Hand & Foot Syndrome (1)		PR
Level2	7	Fatigue (1)		PD
	8	Fatigue (2), Nausea (2)		PR
	9			PD
Level3	10	Fatigue (1), Stomatitis (1), Diarrhea (1)		SD
	11	Nausea (2)		CR
	12	Nausea (2), Fatigue (2), Neutropenia (1)		PR
Level4	13	Diarrhea (1), Fatigue (1)		PR
	14	Neutropenia (2)	CPT skip	PR
	15	Nausea (2), Rash, Diarrhea (2)	CPT skip	SD
	16	Nausea (2), Fatigue (1)	CPT skip	SD

( ): grade, DLT: dose limiting toxicity, CPT: irinotecan
CR: complete response, PR: partial response, SD: stable disease, PD: progression disease.

No DLT occurred in 3 additional patients enrolled at level 1. No DLT was observed at levels 2 or 3. Two of the first 3 patients at level 4 had to skip the third administration of CPT-11 due to grade 2 neutropenia (patient no. 14) and grade 1 diarrhea (patient no. 15), thus level 4 was defined as the MTD. According to the decision criteria for RD described in Patients and Methods, level 4 was therefore considered as the RD of this metronomic chemotherapy.

Tumor response

One patient at level 3 achieved a complete response (CR). Two patients at level 1, 1 at level 2, 1 at level 3, and 2 patients at level 4 achieved partial responses (PR). Three patients did not respond to chemotherapy and the disease progressed (PD); 6 patients showed a stable condition (stable disease: SD). The objective response rate was 43.8% with a 95% confidence interval (95% CI) ranging from 19.8 to 70.1% (Table 3).

Treatment continuation

The median number of treatment cycles in these patients was 4, with range of 2 to 8 cycles. The treatments were stopped for tumor progression in 9 patients, patient refusal in 2, conversion to surgery in 2, complete response in 1, and for adverse effects in 2 patients. The adverse effects leading to treatment discontinuation were diarrhea in the first (grade 3) and second (grade 2) cycle (patient no. 2) and intestinal bleeding in the second cycle (patient no. 15), respectively. Eight of 9 patients whose disease progressed, and 1 of 2 patients whose adverse effects disrupted the treatment, received second-line chemotherapies such as FOLFOX.

DISCUSSION

It has been shown that administration of peroral fluoropyrimidines on a daily schedule for an extended period of time may inhibit growth of the endothelium continuously without inducing drug resistance [20]. However, when bi-weekly or tri-weekly high-dosage CPT-11 is combined with peroral fluoropyrimidines, the combination therapy may often have to be temporarily suspended, which prevents the benefits of peroral fluoropyrimidines from being realized. The type of serious toxic effects, including neutropenia and diarrhea, often associated with a bi-weekly regimen FOLFIRI (irinotecan combined with bolus plus infusion 5-FU/LV), which is one of the standard therapies for metastatic colorectal cancer, were rarely observed

in the present study using low-dose metronomic weekly-CPT-11 and daily S-1. The lower toxicity of our metronomic regimen should be confirmed in a further phase Ⅱ study, as compared with the regimen consisting of tri-weekly high dose CPT-11 and S-1 reported previously [14], or FOLFIRI.

The present study suggested that 60 mg/m^2 of CPT-11 and 80 mg/m^2 of S-1 were the RD for further metronomic chemotherapy study. Metronomic chemotherapy by definition should be continued without temporary suspension due to therapy-related toxic reactions. Therefore, it is reasonable to consider skipping of CPT-11 administration or several-days suspension in S-1 administration as a DLT using conventional dosage escalation criteria. If a skip in CPT-11 administration or a several-day suspension in S-1 administration were excluded as a DLT, a higher dosage than 60 mg/m^2 of CPT-11 might be considered the MTD. Such a higher dosage of CPT-11 may often result in a skip in the CPT-11 administration or interruption in S-1 administration, in this combination therapy of S-1 and CPT-11.

Metronomic chemotherapy has been summarized by Kerbel et al. [16] as showing that 1) conventional cytotoxic anticancer drugs have antiangiogenic effects which could contribute to their efficacy, 2) the antiangiogenic effects of chemotherapy seemed to be optimized by administering such drugs 'metronomically' – in other words in small dosages on a frequent schedule (daily, several times a week, or weekly) in an uninterrupted manner, over a relatively long period, 3) conventional chemotherapy, which is administered at more toxic MTD, requires 2- to 3-week breaks between successive cycles of therapy (which seems to counteract the potential for sustained therapeutically effective antiangiogenic effects), 4) in preclinical models, metronomic chemotherapy can be effective in treating tumors in which the cancer cells have developed resistance to the same chemotherapeutics in a MTD administration (which also has the advantage of being less acutely toxic, therefore making more extended treatments possible), 5) the efficacy of metronomic chemotherapy can be significantly increased when administered in combination with antiangiogenic drugs, such as antibodies against vascular endothelial growth factor (VEGF) or VEGF receptor 2, and 6) some metronomic chemotherapy regimens induce sustained suppression in circulating endothelial progenitor cells and increase the levels of the endogenous angiogenesis inhibitor thrombospondin-1, both of which can suppress neovascularization. However, no one yet knows an adequate dosage or definition of the dosage based on the concept of metronomic chemotherapy. This is a key reason why metronomic chemotherapy is not widely adopted in clinical trials. One possible means of determining the RD in metronomic chemotherapy might be to monitor the circulating endothelial cells and endothelial progenitor cells, which are known to be predictors for the effectiveness of antiangiogenic therapy [21,22].

It was interest that tumor responses were observed constantly at all therapy levels of our metronomic chemotherapy, even at the lowest-dosage of CPT-11. The findings suggested that this metronomic chemotherapy can be expected to have an antiangiogenic effect through continuous inhibition in endothelial cells, in addition to showing an antiproliferating effect against the tumor cells. With regard to antiangiogenic effects, in a preclinical study the 'doublet' combination metronomic chemotherapy using two oral drugs, UFT and cyclophosphamide, where the biologic optimal dosages were determined by effects on levels of circulating endothelial progenitor cells, showed a remarkable prolongation of survival with no evidence of overt toxicity despite long term continuous therapy compared to the monotherapy for advanced metastatic breast cancer [23]. Thus, when antitumor efficacy of metronomic chemotherapy is evaluated in phase Ⅱ and Ⅲ studies it is important to assess the duration of treatment, progression free survival, overall survival and the correlation with circulating endothelial cells and endothelial progenitor cells as surrogate markers for antiangiogenic therapy in addition to the tumor shrinkage (response rate).

Another advantage of our regimen is the schedule of drug administration. Previous *in vitro* studies have shown that CPT-11 down-regulated thymidylate synthase expression in tumor cells, leading to synergy between CPT-11 and 5-FU that was maximal when CPT-11 was given 24 h prior to 5-FU [24,25]. The administration of CPT-11 followed by S-1 with a 2-day interval in our regimen seems to be reasonable, in terms of the antiproliferation effects and of gastrointestinal toxicity.

In conclusion, 60 mg/m^2 of CPT-11 and 80 mg/m^2 of S-1 were found to be the RD for a further phase Ⅱ study of metronomic chemotherapy consisting of weekly CPT-11 and daily S-1. Our new criteria, including a skipping or interruption of drug administration as a DLT in this dosage-escalating study may be useful for indicating the optimal dosages in metronomic chemotherapy.

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Original Contribution

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Thrombocytopenia, an Important Interfering Factor of Antiviral Therapy and Hepatocellular Carcinoma Treatment for Chronic Liver Diseases

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Summary: In patients with chronic liver diseases, thrombocytopenia is a common manifestation which interferes with antiviral therapy for hepatitis C virus (HCV), and with hepatocellular carcinoma (HCC) treatment. While thrombopoietin-receptor agonist is expected to improve thrombocytopenia for patients with chronic liver diseases in 2-3 weeks, there is still a lack of fundamental data about short-term variations in the natural course of platelet count in cirrhotic patients, and the impact of thrombocytopenia on antiviral therapy for HCV-infected patients and patients being treated for HCC. The aims of this study are to investigate sequential changes in platelet count and the impact of thrombocytopenia on antiviral therapy and HCC treatment in patients with chronic liver diseases. A total of 726 chronic liver disease patients were enrolled in this study. Changes of platelet count were examined during a 4-week follow-up. Risk of discontinuation or reduction of peginterferon dosage was evaluated in HCV patients with moderate thrombocytopenia (5-10$\times10^4/\mu$L). Risk of platelet transfusion or splenectomy was evaluated in HCC patients with severe thrombocytopenia ($<5\times10^4/\mu$L). No significant changes of platelet count were observed in cirrhotic patients with thrombocytopenia during a 4-week follow-up. The rate of discontinuation or reduction in dosage of peginterferon was 85.2% (23/27) in patients with moderate thrombocytopenia. Risk of discontinuation or reduction of peginterferon dosage was 3.4-times higher in HCV patients with thrombocytopenia than in those without thrombocytopenia. In HCC patients with severe thrombocytopenia, the frequency of platelet transfusion or splenectomy during HCC treatment was 57.9% (22/38). Risk of platelet transfusion or splenectomy in HCC patients with thrombocytopenia was 57.9-times higher than in those without thrombocytopenia. In conclusion, we demonstrated no significant variation in the short-term natural course of platelet count in cirrhotic patients. In chronic liver disease patients with moderate and severe thrombocytopenia, about 85% of patients treated with peginterferon, and 60% of patients receiving HCC treatments suffered from thrombocytopenia-related limitations, respectively.

Key words eltrombopag, thrombocytopenia, peginterferon, hepatocellular carcinoma, liver cirrhosis

INTRODUCTION

Thrombocytopenia is a common manifestation in patients with chronic liver diseases [1,2]. Thrombocytopenia is also an exclusion criterion for antiviral therapy in patients with chronic hepatitis C virus (HCV) infection [3]. Development of thrombocytopenia during antiviral therapy also causes discontinuation or reduction of dosage [3,4]. In addition, severe thrombocytopenia interferes with hepatocellular

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Abbreviations: ALT, alanine aminotransferase; CRP, C-reactive protein; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; SVR , sustained virologic response.

carcinoma (HCC) treatment [5]. Severe thrombocytopenia has generally been considered a contraindication for hepatectomy [6]. Thus, thrombocytopenia is an important factor that can interfere with clinical treatments in patients with chronic liver diseases. Splenectomy or partial splenic embolization is generally considered an effective therapeutic approach for thrombocytopenia [7-10]. However, these therapeutic procedures are invasive and are not always an option for patients with advanced chronic liver diseases like liver cirrhosis.

There are various theories about thrombocytopenia in chronic liver diseases. Portal hypertension, hypersplenism and bone marrow suppression are factors associated with thrombocytopenia [11,12]. In patients with HCV infection, direct megakaryocyte suppression and antibody-mediated platelet destruction are also involved in the development of thrombocytopenia [12]. In addition, decreased production of thrombopoietin, a hematopoietic growth factor, was recently found to be a causative factor associated with thrombocytopenia in patients with chronic liver diseases [13,14]. Moreover, both interferon and chemotherapy induce decreased platelet count through down-regulation of thrombopoietin production [15,16] and capture of platelets by liver [17,18].

Several new drugs for thrombocytopenia are now under development [19]. A new orally administered thrombopoietin-receptor agonist named eltrombopag increases platelet production through induction of proliferation and differentiation of megakaryocytes *in vivo* [20]. In a phase 1 clinical study of eltrombopag, platelet number began rising at day 5 and peaked at day 15 in healthy male subjects [21]. Results from a randomized placebo-controlled clinical trial showed eltrombopag increased platelet counts in a dose-dependent manner in patients with chronic immune thrombocytopenic purpura [22]. The effectiveness and safety of eltrombopag on thrombocytopenia were demonstrated in a phase Ⅱ clinical trial in 74 patients with liver cirrhosis [23]. After 4 weeks of treatment platelet counts significantly increased in a dose-dependent fashion. Thus, eltrombopag is the first thrombopoietic drug to safely and consistently increase platelet count in patients with liver cirrhosis. However, there are several issues which need to be considered and clarified before eltrombopag can be generally prescribed and administered to patients with chronic liver diseases. Among these is the question of short-term sequential changes in platelet count in cirrhotic patients. There is currently no available data on weekly changes in platelet count, and therefore it is important to clarify the natural course of platelet count in cirrhotic patients. Another issue is the incidence of thrombocytopenia-related limitations during antiviral therapy and HCC treatment. This type of data will provide basic information for development of new drugs against thrombocytopenia.

The aims of the present study were to investigate 1) short-term sequential changes of platelet count in cirrhotic patients and 2) the impact of thrombocytopenia on antiviral therapy and HCC treatment in patients with chronic liver diseases.

MATERIALS AND METHODS

Subjects

A total number of 726 patients with chronic liver diseases were enrolled in the study. For investigation of short-term sequential changes of platelet count, a case-series study was conducted. Twenty-four (24) patients with liver cirrhosis (age 67.4±10.7; male/female=12/12; HCV-related, n=17; hepatitis B virus-related, n=2; alcoholic, n=1; cryptogenic, n=4) were enrolled via consecutive entry. All patients were hospitalized for treatment of ascites, hepatic encephalopathy, or diabetes mellitus. In order to eliminate treatment effects on platelet count, patients who were treated with interferon, radiofrequency ablation, transarterial chemoembolization, chemotherapy, endoscopic variceal ligation, or endoscopic injection sclerotherapy were excluded. Patients with bacterial infection were also excluded, because inflammation affects platelet count [24].

For investigation of risks of thrombocytopenia-related limitations during antiviral treatment, 190 patients with chronic HCV infection were enrolled. Inclusion criteria were i) antiviral treatment aiming at elimination of HCV, ii) HCV genotype 1b, and iii) HCV viral load >2.0 log IU/mL or >100 KIU/mL. Patients who were receiving low-dose peginterferon for prevention of HCC development were excluded.

For investigation of risks of thrombocytopenia-related limitations during HCC treatment, 512 patients with HCC treated by radiofrequency ablation, transcatheter arterial chemoembolization or hepatic arterial infusion chemotherapy (Child-Pugh grade A, n=371; grade B, n=120; grade C, n=21) were enrolled. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, 1984, and the Declaration of Tokyo, 1975 as reflected in a prior approval by the institutional review committee.

Laboratory determinations

Venous blood samples were taken in the morning after a 12-hour overnight fast. Platelet count, hemoglobin levels, white blood cell count, prothrombin time, and serum levels of alanine aminotransferase (ALT), albumin, total bilirubin, and C-reactive protein (CRP) were measured using standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital) as previously described [25].

Short-term sequential changes of platelet count

During a 4-week follow-up period in patients with liver cirrhosis, sequential changes of platelet count were examined in moderate (5 to $10\times10^4/\mu$L of platelet count) and severe thrombocytopenia groups ($<5\times10^4/\mu$L of platelet count) as previously described [26,27]. Platelet count and biochemical parameters were examined once a week for 4 weeks.

Incidence and risk of thrombocytopenia-related limitations during antiviral treatment for HCV

Risk of discontinuation or reduction of peginterferon dosage (peginterferon alfa-2a or peginterferon alfa-2b combined administration with ribavirin) was evaluated retrospectively in HCV patients with moderate thrombocytopenia over their entire treatment period. None of the patients discontinued or reduced peginterferon dosage because of socioeconomic conditions.

Incidence and risk of thrombocytopenia-related limitations during HCC treatment

Inclusion criteria for platelet transfusion and splenectomy were a decrease in platelet count ($<3\times10^4/\mu$L) or an aggravation of hemorrhagic status, such as subcutaneous hemorrhage and gingival hemorrhage. Risk of platelet transfusion or splenectomy during HCC treatment was evaluated retrospectively in patients with severe thrombocytopenia. Patients treated with splenectomy for interferon therapy after HCC treatment were also included.

Statistical analysis

All data are expressed as mean±SD for continuous variables. Statistical significance of changes in parameters during a 4-week period was analyzed by using the Friedman test. Statistical significance of risks of thrombocytopenia-related limitations during interferon and HCC treatments was analyzed by chi-squared test. P values <0.05 were considered significant.

RESULTS

Sequential changes in platelet count

During a 4-week natural course follow-up, there was no significant change in platelet count in any of the patients (Table 1). Serum levels of ALT, albumin, total bilirubin, CRP, and prothrombin time also showed no significant changes in any patients (Table 1). In a stratified analysis, no significant change of platelet count was observed in either the moderate thrombocytopenia group or the severe thrombocytopenia group during a 4-week natural course follow-up (Fig. 1). Levels of other biochemical parameters were not significantly different during a 4-week follow-up in either the moderate thrombocytopenia group or the severe thrombocytopenia group (data not shown).

Incidence and risk of thrombocytopenia-related limitations during interferon treatment

Moderate thrombocytopenia was observed in 14.2%

TABLE 1.
Natural course of biochemical parameters in 24 patients with liver cirrhosis

	Normal range	Week (s) after admission					P
		0	1	2	3	4	
Platelet count ($\times10^4/\mu$L)	13.0 – 36.0	6.2±2.3	5.7±2.0	6.0±2.3	6.1±2.4	6.1±2.3	0.1536
Alanine aminotransferase (U/L)	31 – 33	42±29	37±26	35±19	55±49	37±27	0.5176
Albumin (g/dL)	4.0 – 5.0	2.9±0.7	2.9±0.6	3.0±0.6	3.0±0.4	3.1±0.4	0.5759
Total bilirubin (mg/dL)	0.30 – 1.20	2.0±1.2	1.8±1.2	1.8±1.2	1.7±1.0	1.6±1.0	0.0802
Prothrombin time (%)	60 – 130	70±18	65±17	68±18	69±16	73±20	0.7387
C-reactive protein (mg/dL)	<0.04	0.8±1.3	1.0±1.5	1.1±1.7	0.9±1.4	1.4±2.6	0.8857

Note. Data are expressed as mean ± SD. Statistical significance was analyzed by the Friedman test.

(27/190) of HCV patients receiving combined peginterferon and ribavirin treatment in this study (Table 2). The incidence of discontinuation or reduction in dosage of peginterferon was 85.2% (23/27) due to progression to severe thrombocytopenia ($<5\times10^4/\mu$L). This rate was significantly higher than the incidence of 62.6% (102/163) observed in patients with minor thrombocytopenia or normal range (Table 2). The odds ratio was 3.4. Although the sustained virologic response (SVR) rate was 29.6% in patients with moderate thrombocytopenia, there was no significant difference in SVR rate between patients with and without moderate thrombocytopenia (Table 3).

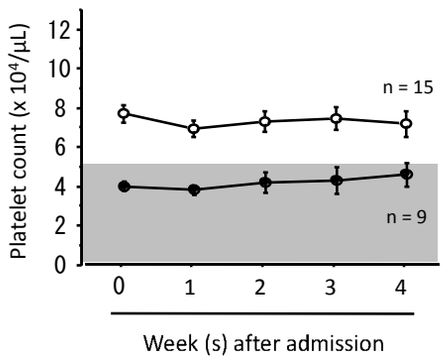

Fig. 1. Sequential measurements of platelet count in cirrotic patients with thrombocytopenia. Moderate thrombocytopenia is indicated as "○" and severe thrombocytopenia is indicated as "●". Values are expressed as mean ± SD. Statistical significance of changes in parameters during a 4-week period was analyzed by using the Friedman test.

Incidence and risk of thrombocytopenia-related limitations during HCC treatment

Severe thrombocytopenia was observed in 3.2% (12/371), 19.2% (23/120), 14.3% (3/21) of HCC patients in Child-Pugh grade A, B, and C groups, respectively. The incidence of platelet transfusion or splenectomy during HCC treatment was 57.9% (22/38) in patients with severe thrombocytopenia. This was significantly higher than the incidence of 2.4% (11/463) seen in HCC patients without severe thrombocytopenia (Table 4). The odds ratio was 57.9.

TABLE 2.
Effects of mild thrombocytopenia before treatment on discontinuation or reduction of peginterferon in patients with chronic hepatitis C

	Discontinuation or reduction		Number of Patients	Odds Ratio	P
	Yes	No			
Platelet count $<10\times10^4/\mu$L	23	4	27	3.439	0.027
Platelet count $\geq10\times10^4/\mu$L	102	61	163		
Number of patients	125	65	190		

Note. Data are expressed as percentage of the total number of patients and number of patients of each category. Statistical significance was analyzed by chi-squared test.

TABLE 3.
Effects of mild thrombocytopenia on sustained viral response rate in patients with chronic hepatitis C

	Sustained virologic response		Number of Patients	Odds Ratio	P
	Yes	No			
Platelet count $<10\times10^4/\mu$L	8	19	27	0.494	0.143
Platelet count $\geq10\times10^4/\mu$L	75	88	163		
Number of patients	83	107	190		

Note. Data are expressed as percentage of the total number of patients and number of patients of each category. Statistical significance was analyzed by chi-squared test.

TABLE 4.
Effects of severe thrombocytopenia on platelet transfusion or splenectomy in cirrhotic patients with hepatocellular carcinoma

	Platelet transfution or splenectomy		Number of Patients	Odds Ratio	P
	Yes	No			
Platelet count $<5\times10^4/\mu L$	22	16	38	57.875	<0.001
Platelet count $\geq5\times10^4/\mu L$	11	463	474		
Number of patients	33	479	512		

Note. Patients treated with splenectomy for initiation of interferon therapy is included. Data are expressed as percentage of the total number of patients and number of patients of each category. Statistical significance was analyzed by chi-squared test.

DISCUSSION

This study demonstrated that there were no changes in platelet count during a 4-week natural course follow-up in patients with chronic liver diseases. About 85% of patients receiving thrombocytopenia-related antiviral treatments either had to discontinue or reduce peginterferon dosage due to clinical thrombocytopenia conditions, and 60% of patients receiving HCC treatment were similarly affected by thrombocytopenia-related limitations.

Although platelet count is unstable and is known to decrease with disease progression in cirrhotic patients [28], no data is available for short-term sequential changes in platelet count in cirrhotic patients. After treatment with eltrombopag, a thrombopoietin-receptor agonist, platelet number begins rising at day 5 and peaks at day 15 [21]. Thus, it is important to clarify the natural course of platelet count in cirrhotic patients before eltrombopag can be generally administrated to patients with chronic liver diseases. In this study, we first demonstrated that platelet count had no significant variation during a 4-week follow-up in patients with liver cirrhosis. Because invasive treatments may affect platelet count, as observed in a previous study [29], hospitalized cirrhotic patients receiving non-invasive treatments were selected for investigation of short-term sequential changes of platelet count. As hepatic inflammation and liver function are also known to affect platelet count [28], laboratory biochemical and liver function parameters were also examined and no significant changes in ALT, albumin, total bilirubin, CRP levels, and prothrombin time were found during a 4-week period. Excluding these factors, the absence of a significant change in platelet count among cirrhotic patients in this study likely reflects the natural course of this disease. Considering that one possible reason for thrombocytopenia is decreased thrombopoietin production in patients with chronic liver diseases [14], administration of eltrombopag, a thrombopoietin-receptor agonist, is expected to be helpful in treating patients with chronic liver diseases at various stages.

Thrombocytopenia can prevent antiviral treatment in patients with chronic HCV infection, however little information is available regarding this issue. In our study, the risk of discontinuation or reduction of peginterferon dosage was about 85% in patients with moderate thrombocytopenia. This was significantly higher than that in patients without moderate thrombocytopenia. Similarly, dose modifications of peginterferon are required in about 20% of patients with no thrombocytopenia [30]. Interferon decreases platelet count through suppression of differentiation in megakaryocytes [16] and capture of platelets by liver [17,18]. A novel thrombopoietin mimetic improves interferon alpha-induced thrombocytopenia *in vivo* [16]. In humans, eltrombopag increases platelet count in cirrhotic patients with HCV infection. Thus, eltrombopag may reduce the risk of discontinuation or reduction of peginterferon dosage in HCV patients.

Although no significant association between moderate thrombocytopenia and SVR rate, which is the rate of continued undetectable serum HCV-RNA 6 months after the completion of anti-viral treatment, was found in our study, Backus et al reported that decreased platelet count is an independent negative predictor for SVR rate in HCV patients treated with peginterferon and ribavirin [31]. The reason for this discrepancy is unclear. However, one possibility is the effect of confounding factors for continuation of

peginterferon. In our study, other factors associated with SVR rate such as sex, age, hepatic fibrosis, and insulin resistance were not matched between patients with and without moderate thrombocytopenia. Thus, these factors may account for the discrepancy between our data and the previous report. Another possibility is differences in administration strategy for peginterferon and ribavirin. In the other study the treatment period was 48 weeks and overall SVR rate was 20.5%. On the other hand, our treatment period was up to 72 weeks and SVR rate was 29.6% even in patients with moderate thrombocytopenia. Thus, prolonged treatment period might have improved the SVR rate, thus eliminating the association between moderate thrombocytopenia and SVR rate.

HCC is now treated with invasive therapies such as resection and radiofrequency ablation, and non-invasive therapies such as chemotherapy [32]. In either case, severe thrombocytopenia seems to adversely affect patient tolerance of these HCC treatments. However, the real incidence of thrombocytopenia-related limitation for HCC treatments is unclear. In our study, about 60% of HCC patients with severe thrombocytopenia received platelet transfusion or splenectomy to improve their thrombocytopenia status. This proportion may be higher than that in other general institutions, because our hospital is a government designated oncology center specializing in HCC. Therefore, we have a relatively high proportion of severe end stage patients desiring intensive care. Although splenectomy and arterial splenic embolization are efficient methods for treating HCC patients with severe thrombocytopenia [7-10], these treatment options are not always available for patients with advanced liver cirrhosis. Thus, severe thrombocytopenia is still a major unresolved issue affecting HCC treatment, and eltrombopag, a thrombopoietin-receptor agonist, is expected to improve HCC treatment.

In conclusion, we demonstrated no change in platelet count during a 4-week natural course follow-up in patients with liver cirrhosis. In patients with moderate and severe thrombocytopenia, about 85% of patients treated with peginterferon and 60% of patients receiving HCC treatment suffered from thrombocytopenia-related limitations, respectively. Since little information is available about short-term sequential changes in platelet count and thrombocytopenia-related limitations on interferon and HCC treatment, this report will provide fundamental information useful when considering administration of eltrombopag to patients with chronic liver diseases.

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Original Contribution

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Effect of Short-term Exposure to Whole Body Vibration in Humans: Relationship between Wakefulness Level and Vibration Frequencies

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Summary: The purpose of this study was to clarify the influence of different vibration frequencies on wakefulness level. Subjects were 7 healthy male university students aged 21.9±1.6 years (mean). All students were non-smokers. Three exposure conditions were used (10 Hz vibration, 20 Hz vibration, and no vibration). Whole-body vertical vibration was applied to subjects sitting on a car passenger seat using a whole-body vibration shaker (CV-300, Akashi) at a single frequency (10 or 20 Hz) at an acceleration level of 0.3 ms^{-2} r.m.s. for 24 min. The objective wakefulness level based on EEGs was evaluated in terms of the alpha attenuation coefficient (AAC) obtained by the Alpha Attenuation Test (AAT). As parameters of psychological stress, salivary 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA) were used. The subjective wakefulness level was evaluated using a questionnaire based on the Kwansei Gakuin Sleepiness Scale (KSS), which is a scale developed for the Japanese based on the Stanford Sleepiness Scale (SSS). The KSS score, representing the subjective wakefulness level, decreased after the exposure irrespective of the exposure condition, but the decrease was not significant. The AAC, representing the objective wakefulness level, significantly decreased only after vibration exposure (10 Hz/20 Hz) but did not differ between the two vibration frequencies. No significant changes were observed after exposure to whole-body vibration in MHPG or HVA as parameters of vibration-related stress. The AAC decreased after exposure to whole-body vibration (10 Hz/20 Hz), suggesting a decrease in the wakefulness level. However, no differences were observed in the influence of the two different vibration frequencies test.

Key words acute effect, electroencephalogram, wakefulness level, whole-body vibration

INTRODUCTION

In the context of work environments, whole-body vibration refers to the rhythmic vibratory movements transmitted to the human body through the seats and floor of cars, trucks, railroad cars, or construction machines. Whole-body vibration is considered to affect health due to the synchronization and amplification of the complex vibration frequencies by the body. Long-term exposure to whole-body vibration has been reported to increase the risk of lower back pain in people operating industrial machines [1-4]. On the other hand, suitable vibration can produce a positive effect on the human body. Several studies have suggested an association between whole-body vibration in public transportation and recovery from fatigue after driving or sedative effects on neonates [5,6]. These findings suggest that whole-body vibration influences the autonomic and central nervous system.

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Abbreviations: AAC, alpha attenuation coefficient; AAT, Alpha Attenuation Test; MHPG, 3-methoxy-4-hydroxyphenylglycol; HVA, homovanillic acid; KSS, the Kwansei Gakuin Sleepiness Scale; SSS, the Stanford Sleepiness Scale.

Factors determining the influences of vibration on the human body are the amplitude, frequency, direction and duration of exposure to vibration. Concerning the relationship between vibration frequency and the resonance frequencies of different organs in the human body, frequency-dependent effects have been reported in digestive organs (4-5 Hz) and the spinal cord (3-5 Hz) [7]. However, whether vibration frequency characteristics are associated with a decrease in the wakefulness level has not been determined. Previous studies have suggested a decrease in the wakefulness level of train drivers due to fatigue caused by their work environment [8,9], and it is hypothesized that physical stimulation by whole-body vibration affects the activity of the autonomic and central nervous system.

We previously reported [10] a decrease in the wakefulness level due to short-term exposure to whole-body vibration using electroencephalogram (EEG) recordings, but did not evaluate differences in this level using varying frequencies or exposure times. In addition, EEG recordings alone are not a sufficient parameter for evaluating wakefulness level. The psychological stress parameters 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA) reflect the kinetics of central catecholamine activity. Therefore, they can be considered as physiological parameters of the wakefulness level, and may be useful for evaluating the influences of vibratory movements on the central nervous system.

We hypothesized that vibration frequency characteristics affect the wakefulness level in whole-body vibration environments. In this study, the influence of the vibration frequency characteristics of whole-body vibration on the wakefulness level was evaluated by measuring subjective and objective parameters of the wakefulness level, and concentrations of MHPG and HVA in saliva as parameters of vibration-related stress.

METHODS

Subjects

The subjects comprised 7 healthy male university students aged 21.9±1.6 years (mean ± SD). They were 169.2±4.2 cm tall and weighed 66.1±12.1 kg. All students were non-smokers. No differences were observed among the 3 exposure conditions with regard to sleep time on the day before the experiment or the postprandial period before tests on the day of the experiment (Table 1).

Methods

The subjects were instructed to spend the 3 days prior to the experiment as usual, avoiding excessive drinking and insufficient sleep. They were also instructed not to consume caffeine-containing foods after lunch on the day of the experiment and to have commercially available solid food (Calorie Mate: 200 kcal) as a light meal at 17:00. All experiments were initiated at 20:00 because of expected intra-day variations in the wakefulness level. All participants were subjected to 3 experimental conditions (whole-body exposure to 10 Hz vibration, 20 Hz vibration, and no vibration) on 3 different days. The order of the 3 conditions was randomly determined to avoid order-related influences. The median (25%-75%) interval between different experiments was 6 days (3.7-16). The experimental schedule is shown in Fig. 1.

Whole-body vertical vibration was applied for 24 min to subjects sitting in the passenger seat of a car using a vibration shaker (ASE-385; AKASHI, Japan). The vibration was applied at a single frequency (10 or 20 Hz) and an acceleration level of 0.3 ms^{-2} r.m.s. according to ISO 2631-1-1 [11], based on field measurement data of actual exposure on bulldozers in a work environment. The subjects were instructed not to use the back support during exposure to whole-body vi-

TABLE 1.
Status of the subjects before the experiments

	WBV (–)	WBV (+)	
		10Hz	20Hz
Age (yr)	21.9±1.6		
BMI (Body Mass Index)	23.1±3.7		
Sleeping time (hour)	7.7±2.0	7.1±0.6	6.8±1.4
Time to previous meal (hour)	2.6±0.7	2.4±0.5	2.7±0.8

(Mean±SD)

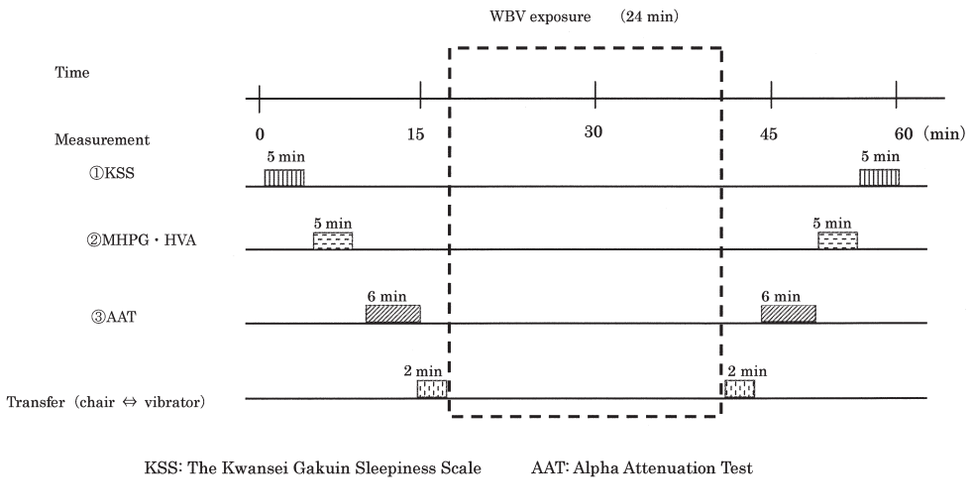

Fig. 1. Experimental schedule of the measurements in this study.

bration. Vibration acceleration was maintained at a constant level using a vibration level meter (VM-52, RION).

EEGs were recorded after the application of electrodes following the international 10-20 method (C3-A2, O1-A2, O2-A1). To reduce electric resistance, cutaneous sebum was adequately removed using a polisher before electrode application. EEGs were continuously recorded during the experimental period. The subjects maintained a relaxed posture on a sofa with a support for the back and armrest before and after exposure to whole-body vibration, while staring at a black dot (3.0 cm in diameter) placed 2.0 m in front of them. After electrode application, the subjects were interviewed regarding sleep time and activities performed during the previous 3-day period prior to the experiment and their physical condition on the day of the experiment.

Salivary MHPG and HVA were used as psychological stress parameters. Saliva was collected by applying a cylindrical cotton swab to the oral cavity for 2 min and using a conical centrifugation tube for saliva collection (Salivette) before and after exposure to whole-body vibration. The collected saliva was returned to the conical tube, centrifuged for 3 min, and stored at −20°C until measurement. After deproteinization of both MHPG and HVA with 25% perchloric acid, the fluorescence of the supernatant obtained after centrifugation was measured by HPLC at an excitation wavelength of 280 nm and a fluorescence wavelength of 320 nm.

The subjective wakefulness level was evaluated using the Kwansei Gakuin Sleepiness Scale (KSS) questionnaire [12]. This is a scale developed for the Japanese based on the Stanford Sleepiness Scale (SSS) devised by Hoddes in 1972, and consists of 22 items. The mean of the scores for 22 items is assumed to be the total score, and a mean score close to 0 indicates a high wakefulness level, while a mean score close to 7 indicates a low wakefulness level.

EEGs were recorded using an electroencephalograph (Neurofax EEG-8314; Nihon Kohden, Tokyo, Japan). By performing FFT (1 section, 4 sec; sampling frequency, 100 Hz) using a biosignal time series analysis program (Trend Viewer, Kissei Comtec, Nagano, Japan), we calculated the power spectra of the θ (4-7 Hz), α (8-11.8 Hz), and β (14-30 Hz) waves. In addition, we obtained the mean values of power spectra when the eyes were open and when they were closed. In this study, the objective wakefulness level based on EEGs was evaluated in terms of the alpha attenuation coefficient (AAC) obtained by the Alpha Attenuation Test (AAT) reported by Michimori et al. [13,14] The power spectrum of α waves increases when the eyes are closed. When the wakefulness level is high, blocking becomes marked when the eyes open, and the power spectrum of α waves markedly decreases. When the wakefulness level is low, there are few differences in the power spectrum of α waves between when the eyes are open and when they are closed. Based on these findings, the AAC is defined as the mean power spectral value of α waves when the eyes are closed/that when they are open. A higher AAC indicates a higher wakefulness level. The AAC was analyzed using EEG signals obtained from O1-A2.

In AAT, experiments with the eyes open and closed were repeated 3 times for 1 min each in the sitting position according to the instructions of the examiner;

these experiments were performed before and after exposure to whole-body vibration. Measurement of the subjective wakefulness level and saliva collection were performed before and after AAT.

The methods used for experiments where no whole-body vibration was applied were similar to those employed for whole-body vibration, except that there was no exposure to vibration itself. The period corresponding to the whole-body vibration exposure time was spent in the sitting position in the shaker, as in the other experiments. The subjects entered the room for experiments at about 20:00. The room temperature, humidity, background noise level, and illumination in the experimental room were maintained at approximately 21.4±0.8 °C, 50±5%, 64 dB (A), and 510 lx, respectively.

Statistical analysis

Since we could not confirm the normality of the distribution of the subjective wakefulness level (KSS), objective wakefulness level (AAC), or salivary MHPG/HVA (Kolmogorov-Smirnov test), Wilcoxon's signed rank test as a non-parametric method was used for comparison between the presence and absence of whole-body vibration exposure. $P<0.05$ was regarded as significant. Statistical analysis was performed using a statistical software package (SPSS 11.5 J).

Ethical considerations

The subjects were provided with oral and written explanations of the purpose/methods/contents of the investigation. They were also informed that they had the right to refuse participation in this investigation, that the results of the investigation would remain confidential and be used only for this study, and that individuals could be identified only by the research representative. Following this, consent was obtained from all subjects. When the subjects inquired about the contents of the measurement, an adequate explanation was provided.

Whole-body vibration exposure was set at a level that did not affect health according to the ISO 2631-1 standard. We explained to the subjects that their participation in this study had no short- or long-term effects on their health, and that experiments would be immediately discontinued in cases such as the discomfort condition of the subjects. This study was approved by the Ethics Committee on Medicine of Kurume University (Study No. 2562).

Definition of terms

***Amplitude*:** The amplitude of a vibration can be quantified by its displacement, its velocity or its acceleration. For practical convenience the acceleration is usually measured with accelerometers.

***Frequency*:** The frequency of vibration, which is expressed in cycles per second, affects the extent to which vibration is transmitted to the body (e.g., to the surface of a seat), the extent to which it is transmitted thought the body (e.g., from the seat to the head), and the effect of the vibration in the body.

***Direction*:** Vibration may take place in three translational directions and three rotational directions. For seated persons, the translational axes are designated x-axis (fore-and-aft), y-axis (lateral) and z-axis (vertical).

***Duration*:** The period of exposure to vibration

RESULTS

Subjective awareness level

***KSS*:** Changes in the wakefulness level in terms of the KSS score are shown in Table 2. The KSS score increased after the experiment irrespective of whole-body vibration exposure, indicating a decrease in the subjective wakefulness level, but no significant difference was observed.

Objective awareness level

***EEG (AAC)*:** Changes in AAC as a parameter of the objective wakefulness level are shown in Fig. 2. The

TABLE 2.
Changes in subjective wakefulness level as measured by KSS

	WBV (–)		WBV (+)			
			10Hz		20Hz	
	Before	After	Before	After	Before	After
KSS	3.6 (3.6-4.3)	4.5 (4.3-4.9)	3.6 (2.9-4.0)	4.6 (3.6-4.9)	3.1 (2.4-3.5)	4.3 (3.6-4.9)

(median, 25th-75th percentile)

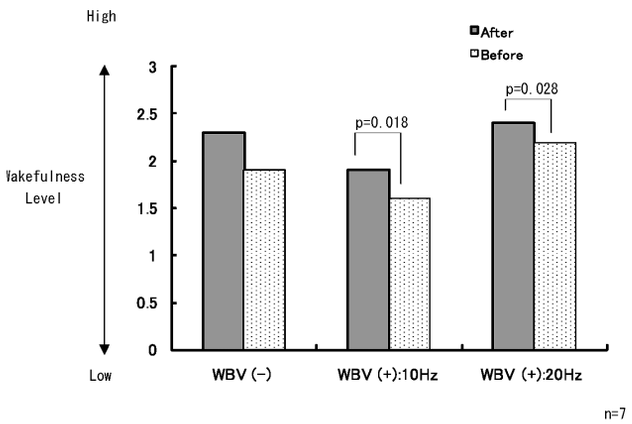

Fig. 2. Changes in objective wakefulness level by AAC

AAC decreased irrespective of the exposure conditions, indicating a decrease in the awareness level, but significant differences were observed only in the presence of exposure to vibration. There was no significant difference between the two frequencies.

***Biochemical examination (MHPG/HVA)*:** Changes in MHPG and HVA under each whole-body vibration exposure condition are shown in Table 3. The MHPG concentration (median) increased after the exposure experiment in the presence of exposure (10 and 20 Hz). The HVA concentration (median) increased in the absence of exposure and in the presence of exposure (10Hz). There was no significant difference among the three conditions.

DISCUSSION

The KSS as a subjective measure of awareness level decreased after the experiment under each exposure condition, but the decreases were not significant. The AAC as an objective measure of awareness level significantly decreased after the experiment only in the presence of exposure (10 and 20 Hz). In addition, no significant change was observed in MHPG or HVA as parameters of psychological stress. Thus, the vibration acceleration level used in this study (0.3 ms^{-2} r.m.s.) affected the awareness level without causing psychological stress. However, since no difference was observed in the responses between the two vibration frequencies (10 and 20 Hz) used in the present study, no specific vibration frequency characteristics could be identified.

Concerning the influences of exposure to whole-body vibration on the wakefulness level, Landström et al. exposed subjects to a single frequency vibration (3 Hz) or random vibration for 15 min at the same acceleration level (0.3 ms^{-2}), and analyzed the wakefulness level, focusing on changes in the power spectra of frequency components of EEGs. They observed a significant increase in the θ wave component and a significant decrease in the α wave component after exposure to vibration, indicating a decrease in the awareness level after exposure [15]. Although the frequency employed in their study differed from those used in ours, their results support ours. We previously reported a decrease in the wakefulness level after exposure to 10 Hz whole-body vibration for a short period (12 min) [10]. The level of exposure to whole-body vibration used in our previous study (vibration acceleration, 0.6 ms^{-2} r.m.s.) was twice the level in this study. Since no psychological stress parameters were measured in our previous study, whether this acceleration level causes psychological stress is unclear. However, even the vibration exposure level used in the present study reduced wakefulness, a result which will be useful when designing future studies to determine the threshold level of vibration exposure needed to reduce the wakefulness level.

In train drivers, a decrease in the wakefulness lev-

TABLE 3.
Changes in median MHPG and HVA by exposure to Whole-body vibration

	WBV (–)		WBV (+)			
			10Hz		20Hz	
	Before	After	Before	After	Before	After
MHPG	17.3 (7.1-33.2)	17.1 (14.2-34.0)	23.8 (18.4-41.0)	30.9 (16.2-37.5)	19.9 (16.8-24.9)	21.6 (15.4-24.9)
HVA	7.4 (3.6-15.2)	10.4 (4.6-14.8)	9.0 (2.9-10.9)	12.7 (5.3-32.0)	9.3 (3.6-12.3)	9.5 (7.5-12.1)

(median, 25th-75th percentile)

el due to fatigue has been suggested [8,9]. However, our results suggest that the wakefulness level is affected not only by fatigue but also by certain direct effects of exposure to whole-body vibration. The vibration acceleration level used in this study (0.3 ms^{-2}) is similar to the general vibration exposure level in work environments involving traveling and operating industrial machines, which suggests that occupational exposure to whole-body vibration can be a risk of a decrease in the awareness level of drivers. Therefore, further detailed evaluation of the relationship between exposure to whole-body vibration and the wakefulness level may be useful for preventing accidents in occupations that involve driving. In the future, it will be necessary to evaluate the influences of whole-body vibration on the human body in terms of the wakefulness level as well as performance, such as changes in the response time and work efficiency under vibration exposure conditions similar to actual driving-based work environments.

The wakefulness level has been reported to be affected not only by vibration exposure but also by exposure to a single noise [16]. In this study, the wakefulness level decreased with time even in the absence of vibration exposure. This may be due to the background noise of 64 dB (A), which could have affected the wakefulness level as a stimulus, reducing the difference in the decrease in the wakefulness level between the presence and absence of exposure to whole-body vibration. The vibration acceleration level in this study was 0.3 ms^{-2}, which is similar to the general vibration exposure level in work environments involving traveling and operating industrial machines. Therefore, it is also possible that this acceleration level induced comfort and relaxation rather than discomfort in the subjects, resulting in a decrease in the wakefulness level. The influences of vibration exposure itself could not be evaluated, probably because of the background noise and the fact that the levels of exposure to whole-body vibration were similar to those experienced in passenger cars in ordinary life. This is supported by the comments of most subjects, who reported that they were comfortable during exposure to whole-body vibration.

AAC, used as an objective parameter of the wakefulness level, is obtained by performing AAT, which was developed by Michimori et al. [13,14]. A correlation has been reported between the AAC and multiple sleep latency test (MSLT) using objective physiological parameters. Unlike MSLT, which requires various experimental conditions, AAC allows estimation of the objective wakefulness level via a simple procedure in a short time, and may therefore be more useful. In this study, we were able to measure AAC in a short period with minimum discomfort; therefore, AAC may be appropriate as a parameter of the wakefulness level during exposure to whole-body vibration. The wakefulness level may be affected by sleep and wakefulness rhythms. In this study, to minimize these influences, experiments were initiated at the same time of day in all cases. The subjects were instructed on adjustments to daily activities, such as maintaining appropriate sleep time from 2-3 days before the day of the experiment. Therefore, the sleep time and postprandial period on the experimental day did not differ among the vibration exposure conditions. However, since experiments were initiated at 20:00, daytime activities may also have affected the results. Although the subjects were instructed to avoid excessive exercise, the degree of fatigue during the daytime may have affected the results. In the future, for a more accurate evaluation of the influences of exposure to whole-body vibration, more detailed adjustments of daily activities may be necessary.

As parameters of psychological stress, the salivary MHPG and HVA concentrations did not differ between before and after the exposure experiment irrespective of the presence or absence of exposure. MHPG and HVA reflect central noradrenergic neuronal activity and are also parameters of stress responses in healthy individuals [17]. The use of conventional biological parameters of stress measured by blood analysis requires blood collection, which can be a stress load itself, inducing vasoconstriction and an increase in blood pressure. Therefore, the correct measurement of stress by this method has been considered difficult. In this study, MHPG and HVA were used as parameters and were measured in salivary samples. Since the collection of saliva causes no physical pain and can be readily performed in a short time, this method was useful. However, the MHPG concentrations observed in this study were higher than those in previous studies. This may be associated with the psychological state of the subjects placed in a special experimental environment, and differences in the measurement method, which was HPLC in this study and gas chromatography/mass spectrometry in the report by Mass et al. [18] and other previous studies. Concerning the absence of changes in MHPG in this study, a previous study showed no activation of the noradrenergic nervous system and no increase in the MHPG concentration in the prefrontal area during a simple addition task [19]. The relatively high concentration of MHPG in this study, therefore, may not be due to the instruction given to the subjects, i.e., to stare at a black dot. Further studies on changes in parameters of psycho-

logical stress should take into consideration psychological influences and work loads.

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Original Contribution

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Azelnidipine Decreases Plasma Matrix Metalloproteinase-9 Levels after Endovascular Abdominal Aortic Aneurysm Repair

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Summary: We investigated the changes of matrix metalloproteinase (MMP) -9 in the peripheral blood samples of patients undergoing endovascular aneurysm repair (EVAR) for abdominal aortic aneurysms (AAAs), and the effect of azelnidipine on plasma MMP-9 levels in those patients. Levels of MMP-9 were measured in 22 patients who underwent EVAR for AAAs, and results were compared between a group receiving 16 mg azelnidipine daily (n=12) and a control group without azelnidipine (n=10). Measurements were taken preoperatively, and at 1 month and 3 months, postoperatively. Patients without endoleaks after EVAR showed a significant decrease in mean plasma MMP-9 levels (preoperative value: 39.5±14.3 ng/mL, after 1 month: 25.0±12.6, after 3 months: 28.2±10.2 ng/mL; P=0.004). In contrast, no significant decreases in mean plasma MMP-9 levels were observed in the patients with endoleaks after EVAR (preoperative value: 37.5±9.0 ng/mL, after 1 month: 26.8±8.4, after 3 months: 38.5±15.7 ng/mL; P=0.219). Moreover, among patients without endoleaks, those receiving azelnidipine showed a significantly greater decrease in the mean plasma MMP-9 levels for 3 months postoperatively (preoperative value: 47.7±13.2 ng/mL, after 1 month: 26.6±12.8, after 3 months: 26.1±11.4 ng/mL; P<0.001) compared with the control group without endoleaks (preoperative value: 31.3±10.5 ng/mL, after 1 month: 33.4±12.1, after 3 months: 30.3±9.1 ng/mL; P=0.792). These results showed that azelnidipine treatment in patients without endoleak after EVAR was associated with a significant decrease in mean plasma MMP-9 levels for 3 months postoperatively.

Key words abdominal aortic aneurysm, calcium channel antagonist, azelnidipine, matrix metalloproteinase-9, endovascular aneurysm repair

INTRODUCTION

Abdominal aortic aneurysms (AAAs) are characterized by destructive remodeling of elastic media and the outer aortic wall, and recent studies have emphasized the disease mechanisms involving chronic aortic wall inflammation and the progressive degradation of fibrillar matrix proteins [1,2]. Matrix metalloproteinase (MMP) -9 plays a pivotal role in connective tissue destruction, and elevated MMP-9 levels in aneurysmal tissue and plasma have been reported [1]. This increase in enzyme level has been implicated in the progression of aneurysmal disease [3]. In addition, it has been shown that the tissue inhibitor of metalloproteinases (TIMP) plays a role in the modulation of MMP activity.

Lorelli et al. [4] reported that plasma MMP-9 levels were significantly decreased in patients without

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Abbreviations: AAAs, abdominal aortic aneurysms; ARB, angiotensin receptor blocker; CFA, common femoral artery; COPD, chromic obstructive pulmonary disease; CT, computed tomography; EVAR, endovascular aneurysm repair; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; TNF-α, tumor necrosis factor-α.

endoleaks after endovascular aneurysm repair (EVAR) for AAAs, while patients with endoleaks did not have a significant decrease in plasma MMP-9 levels 3 months after EVAR. It appears, therefore, that evaluation of plasma MMP-9 can be implicative for endoleaks after EVAR, however no clinical data is available about changes in plasma MMP-9 levels after EVAR in Japanese patients.

EVAR for AAAs, which was introduced in 1991, has been shown to be a practicable alternative to open repair in short to medium term follow-up periods. We began using two commercial medical devices (Cook Zenith and Gore Excluder) for minimally invasive EVAR at our institute in 2006.

The athero-protective effects of azelnidipine, a calcium antagonist, are most likely not due to its calcium channel blocking properties, but rather to its unique antioxidative characteristics [5]. And, since azelnidipine is retained in the vascular wall due to its high lipophilicity, its efficacy for vascular remodeling is superior to that of other calcium channel antagonists [6]. Moreover, it has recently been shown that azelnidipine also suppresses MMP-9 levels and decreases aneurysmal formation in a rat model in our insititute [7]. However, the effects of azelnidipine in patients with AAAs are unknown. Therefore, we investigated the changes of MMP-9 in blood samples of patients undergoing EVAR for AAAs, and evaluated the effect of azelnidipine on plasma MMP-9 levels and aneurysmal shrinkage in those patients.

MATERIALS AND METHODS

All patients who underwent elective EVAR for AAAs between May 2007 and March 2008 at our hospital were enrolled in this study. All patients underwent EVAR after informed consent was obtained in accordance with the Institutional Review Board of Kurume University School of Medicine.

Inclusion criteria were an atherosclerotic infrarenal atherosclerotic true AAA and availability for postoperative follow-up. Exclusion criteria were clinical and/or laboratory suspicion of infection of any site, acute aortic dissection, traumatic aortic lesions, ruptured aneurysms, mycotic aneurysms, chronic renal failure on dialysis, autoimmune diseases, connective tissue disease (Marfan syndrome, Ehlers-Danlos syndrome), aortitis, associated neoplasias, immunodeficiencies, use of anti-inflammatory drugs, chemotherapy or immunosuppressant treatment, clinical and/or tomographic indication for open surgery, and difficulties with respect to regular postoperative follow-up.

The indication of surgical treatment for true AAAs was a diameter over 45 mm in our institution.

The patients were randomly divided into two groups. The azelnidipine group was administered 16 mg of azelnidipine daily, and administration for 1 year after the operation. The control group patients were administered anti-hypertensive drugs not including azelnidipine. We checked postoperative compliance with the azelnidipine protocol at our outpatient clinic during the follow-up period.

All patients underwent a bilateral inguinal skin incision and taping of the bilateral common femoral artery (CFA) under general anesthesia. Under general heparinization (60 units/kg iv), the main body of the endovascular repair device was inserted into the infrarenal abdominal aorta. Another contra body was inserted from the other side of the CFA. EVAR was accomplished with either of two commercially available endovascular stent graft systems (Cook Zenith, Bloomington, Indiana/Gore Excluder, Flagstaff, AZ, USA).

Peripheral venous blood samples were obtained from all patients and were collected by venipuncture into tubes containing sodium ethylenediamine tetraacetate (EDTA). Samples were obtained at admission, at 1 month and at 3 months after EVAR. The blood samples were centrifuged (3000× for 10 min) within 60 min of collection and the plasma obtained from the samples was frozen at −30°C. A commercially available one-step sandwich enzyme-linked immunosorbent assay (ELISA) (SRL, Inc., Tokyo, Japan) was used to determine plasma MMP-9 levels (averaged value of healthy Japanese; 38±13 ng/mL) preoperatively, and at 1 month and 3 months postoperatively [8].

The maximum aneurysm diameter was measured from the outer wall to outer wall by computed tomography (CT) scan preoperatively, at 3 months and at 1 year after EVAR.

Preoperative plasma MMP-9 and maximum AAA diameter were considered the baseline. The percent change was calculated according to the following formula: ([follow-up measurement-baseline measurement] /baseline measurement)×100 (%).

Statistical methods

The plasma MMP-9 levels and the maximum AAA diameters were prospectively compared between the two groups. The data were recorded as the mean ± the standard error of the mean. The unpaired *t*-test was used to compare the two groups with respect to preoperative characteristics, and postoperative mean change and mean percent change of plasma MMP-9 levels.

Preoperative maximum AAA diameters and plasma MMP-9 levels were compared by linear regression analysis. Statistical comparisons between the two groups with regard to patient characteristics such as gender, drugs, and preoperative risk factors were conducted by χ^2 analysis. Significant differences among the mean values of plasma MMP-9 levels and maximum AAA diameters at three points were evaluated by repeated-measures analysis of variance. Statistical significance was assumed at $p<0.05$.

RESULTS

There were 22 patients enrolled in this study, 12 in the azelnidipine group, and 10 in the control group. The patients and preoperative characteristics of both groups were presented in Table 1. There were no positive or negative correlations among the preoperative characteristics between the two groups except for preoperative plasma MMP-9 levels (P=0.009). There was no relationship between preoperative plasma MMP-9 levels and maximum AAA diameter (r=−0.074 P=0.741) in any cases. There were also no significant differences in drug treatment (except for calcium channel antagonists) and blood pressure between the two groups (Tables 2 and 3). Moreover, no side effects were observed in the azelnidipine group, such as tachycardia, leg edema, hypotension, palpitation, etc.

Thirteen Cook Zenith (7 cases in azelnidipine group and 6 in control group) and 9 Gore Excluder endografts (5 cases in azelnidipine group and 4 in control group) were used for EVAR for AAAs. There were no hospital deaths. EVAR was successfully performed in all patients, and there was no conventional open repair conversion. Type Ⅱ endoleaks caused by retrograde flow from lumbar arteries were evident on fol-

TABLE 1.
Patients and preoperative characteristics

	Azelnidipine group (%)	Control group (%)	P value
No. of patients	12	10	
Age (y)	75.6±6.6	74.7±8.0	0.758
Sex (Male:Female)	10:2 (83.3%:16.7%)	8:2 (80.0%:10.0%)	0.840
Hypertension	11 (91.7%)	7 (70.0%)	0.190
Hyperlipidemia	4 (33.4%)	4 (40.0%)	0.746
Diabetes mellitus	1 (8.3%)	1 (10.0%)	0.892
Ischemic heart disease	4 (33.4%)	3 (30.0%)	0.867
Cerebro vascular accident	3 (25.0%)	2 (20.0%)	0.781
COPD	4 (33.4%)	4 (40.0%)	0.746
AAA diameter (mm)	50.8±4.2	48.8±8.7	0.531
Preoperative plasma MMP-9 (ng/mL)	45.6±12.6	31.4±9.9	0.009

MMP, matrix metalloproteinase; COPD, chronic obstructive pulmonary diseas; AAA, abdominal aortic aneurysm.

TABLE 2.
The comparison of hypotensive agents between two groups

Drug	Azelnidipine group N=12 (%)	Control group N=10 (%)	P value
Calcium antagonist	12 (100.0%)	5 (50.0%)	0.005
β blocker	2 (16.7%)	3 (30.0%)	0.457
Diuretic agent	1 (8.3%)	3 (30.0%)	0.190
Isosorbide dinitrate	1 (8.3%)	1 (10.0%)	0.892
ARB	3 (25.0%)	3 (30.0%)	0.793

ARB, Angiotensin receptor blocker.

TABLE 3.
The time course of blood pressure in each group

Blood pressure	Azelnidipine group	Control group	P value
Pre systolic pressure	134.3±11.3	130.3±21.2	0.311
Pre diastolic pressure	75.8± 7.5	76.1± 8.9	0.921
Pre mean pressure	92.8±10.8	93.0±14.1	0.955
3M systolic pressure	143.4±19.9	132.4±12.4	0.137
3M diastolic pressure	78.1±10.7	77.4±10.3	0.892
3M mean pressure	98.9±15.7	94.8±10.3	0.479
1Y systolic pressure	132.0±26.4	136.3±13.2	0.731
1Y diastolic pressure	71.8±10.3	80.3±15.0	0.343
1Y mean pressure	92.2±16.3	100.3±14.2	0.490

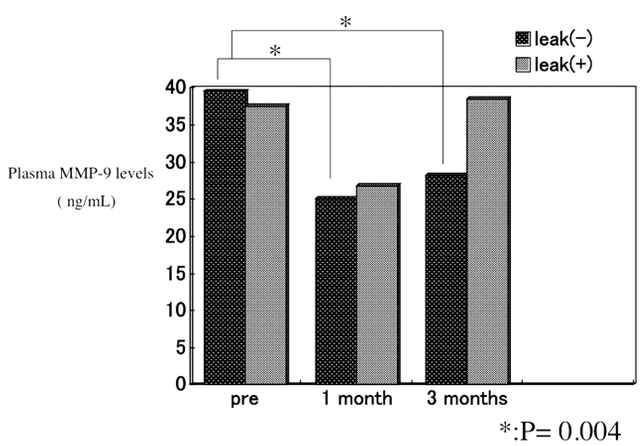

Fig. 1. Comparison of mean plasma MMP-9 levels (ng/mL) over time with or without endoleak for 3 months following surgery. The mean plasma MMP-9 levels were significantly decreased postoperatively in no endoleak case (P=0.004). But, in the patients with endoleak were not observed (P=0.219).

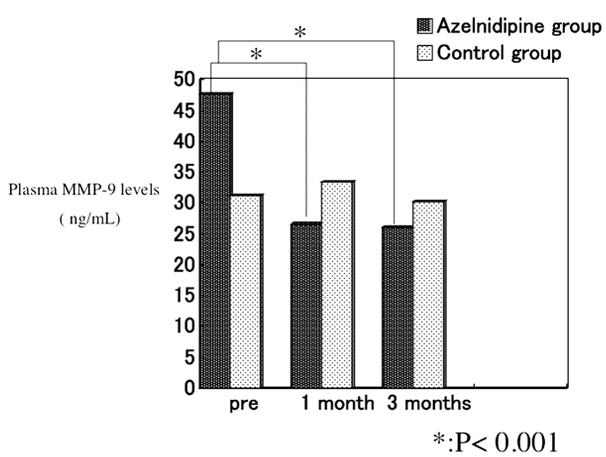

Fig. 2. Comparison of mean plasma MMP-9 levels (ng/mL) in no endoleak case between the two groups. The mean plasma MMP-9 levels (ng/mL) in patients with azelnidipine treatment were significantly decreased postoperatively, in contrast, there was no significant decrease in the control group (azelnidipine group; P<0.001, and the control group; P=0.792, respectively).

low-up CT scan in 18.2% (4/22) of all patients (3 cases in the azelnidipine group and 1 in the control group). There was no enlargement in maximum aneurysm diameter in the endoleak cases, postoperatively.

Among patients with no endoleaks, there was a significant decrease in the mean plasma MMP-9 levels during the study period (preoperative value: 39.5±14.3 ng/mL, after 1 month: 25.0±12.6, after 3 months: 28.2±10.2 ng/mL; P=0.004). In contrast, no significant decreases in the mean plasma MMP-9 levels were seen in the patients with endoleaks after EVAR during the same period (preoperative value: 37.5±9.0 ng/mL, after 1 month: 26.8±8.4, after 3 months: 38.5±15.7 ng/mL; P=0.219) (Fig. 1).

In the azelnidipine group without endoleaks, significant decreases in the mean plasma MMP-9 levels were noted for 3 months after EVAR (preoperative value: 47.7±13.2 ng/mL, after 1 month: 26.6±12.8, after 3 months: 26.1±11.4 ng/mL; P<0.001). But, in the control group without endoleaks, plasma MMP-9 levels did not change significantly (preoperative value: 31.3±10.5 ng/mL, after 1 month: 33.4±12.1, after 3 months: 30.3±9.1 ng/mL; P=0.792) (Fig. 2). These findings demonstrated that azelnidipine treatment af-

ter EVAR in patients without endoleaks was associated with a significant decrease in mean plasma MMP-9. Analysis of the direction of mean percent change in plasma MMP-9 levels revealed that follow-up values at 3 months were decreased an average of 45.2% (mean change, −21.6 ng/mL) in patients with no endoleaks who underwent EVAR with azelnidipine treatment. In contrast, plasma MMP-9 levels fell by an average of 2.1% (mean change, −1.0 ng/mL) in control group patients with no endoleaks. The mean change and mean percent change in plasma were decreased significantly in the azelnidipine group with no endoleaks as compared with the control group with no endoleaks (P<0.001 and <0.001, respectively) (Table 4).

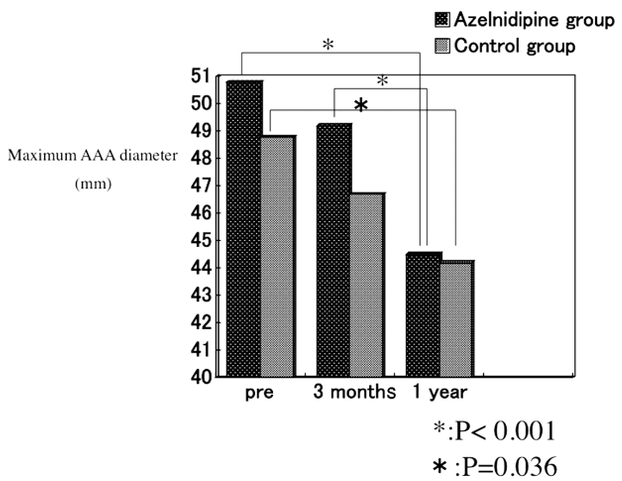

Fig. 3. Comparison of maximum AAA diameters (mm) before and during follow-up after surgery between the two groups. There were significantly decreases in maximum AAA diameters for follow-up periods in each group (azelnidipine group; P<0.001, and the control group; P=0.036, respectively).

Moreover, among overall patients with no endoleaks, the maximum AAA diameters were significantly decreased postoperatively in both groups (azelnidipine group; preoperative value: 50.8±4.2 mm, after 3 months: 49.2±4.3 mm, after 1 year: 44.5±7.5 mm; P<0.001, control group; preoperative value: 48.8±8.7 mm, after 3 months: 46.7±8.0, after 1 year: 44.2±7.6 mm; P=0.036) (Fig. 3). In the azelnidipine group, the maximum AAA diameter at 1-year follow-up fell by an average of 14.4% (mean change, −7.1 mm). In contrast, maximum AAA diameters decreased an average of 7.5% (mean change, −3.7 mm) in patients in the control group, however these differences did not attain statistical significance (P=0.094 and 0.101, respectively) (Table 5).

DISCUSSION

MMP-9 is associated with atherosclerotic arterial remodeling. Elevated MMP-9 levels in aortic aneurys-

TABLE 4.
The comparison of mean change and mean percent change of plasma MMP-9 levels when compared between two groups with no endoleak case

	Azelnidipine group	Control group	P value
Mean change (ng/mL)	–21.6±8.5	–1.0± 8.0	<0.001
Percent change (%)	–45.2±6.1	–2.1±26.5	<0.001

MMP, matrix metalloproteinase.

TABLE 5.
The comparison of mean change and mean percent change of maximum AAA diameters when compared between two groups with no endoleak case

	Azelnidipine group	Control group	P value
Mean change (mm)	–7.1±6.6	–3.7±3.5	0.101
Percent change (%)	–14.4±13.0	–7.5±6.0	0.094

AAA, abdominal aortic aneurysm.

mal tissue and plasma have been demonstrated [3]. MMP-9 has been shown to be the principle elastase within the aneurysmal aortic wall and is excessively expressed by macrophages [3,9]. The enzyme is produced as a result of a cytokine-mediated chronic inflammatory state of unclear cause, which is responsible for macrophage infiltration of the aortic wall.

The overproduction of MMP-9 contributes to the progression of aneurysmal disease by mediating the connective tissue destruction seen in the extracellular matrix [10]. An exponential relationship between the amount of MMP-9 produced in AAA tissue and the plasma MMP-9 levels (r=0.856) has been reported [10]. Moreover, the wall of ruptured AAAs has been shown to be associated with high levels of MMP-9 [11]. Experimental models have shown that the inhibition of these proteinases may actually slow matrix breakdown and limit the growth of aneurysms [12-17]. In healthy tissue, MMP activity is tightly regulated by TIMPs. Imbalance of MMP/TIMP homeostasis is thought to lead to aneurysmal dilatation [18].

Lorelli et al. [4] reported that successful endovascular repair for AAA had resulted in decreased plasma MMP-9 levels at 3 months following surgery. Plasma MMP-9 levels did not change significantly in patients with endoleaks after EVAR. These results suggested that the change in plasma MMP-9 levels might be an indicator of the biologic significance associated with an endoleak. In our study, the mean plasma MMP-9 levels in the patients with endoleaks after EVAR did not significantly decrease postoperatively. These data were in agreement with the earlier reports. To our knowledge, this report is the first to demonstrate changes in plasma MMP-9 levels in Japanese patients who underwent EVAR for AAAs, and to discuss the clinical significance of these changes. However, the number of patients in the present study was too small to allow us to come to a definite conclusion concerning endoleak cases in general.

In our previous study, azelnidipine reduced the expansion of experimental AAAs while decreasing the MMP-2 and MMP-9 levels in a rat model [5]. Moreover, the slopes of the changes of maximum AAA diameters were reported to be significantly different, demonstrating that the rate of aneurysmal shrinkage was faster in patients without an endoleak compared with those patients with an endoleak [19]. Therefore, we studied the efficacy of azelnidipine in suppressing plasma MMP-9 levels and reducing maximum AAA diameters in patients without endoleak.

In the azelnidipine group, the plasma MMP-9 levels were significantly decreased after successful EVAR. Moreover, mean change and mean percent change of the plasma MMP-9 levels showed significantly greater decreases in the azelnidipine group without endoleaks than in the control group without endoleaks. This result appears to be due to the unique characteristics and properities of azelnidipine in AAA patients.

Azelnidipine is recognized to play an important role in vascular remodeling. Azelnidipine is a highly lipid soluble calcium antagonist and is retained in the vascular wall after its clearance from the blood [6]. Several investigators [20,21] have recently shown that NF-κβ activation is required for the maximal induction of MMP-9 transcription in some experimental systems, while others have reported that azelnidipine may act via both anti-inflammatory and antioxidative responses, inhibiting NFκβ as well as the adhesion of mononuclear cells and monocyte chemoattractant protein-1 (MCP-1), which are associated with the production of MMP-9. Enhanced production of the chemotactic cytokines interleukin-8 and MCP-1 has been reported in human AAAs [22]. The inhibition of TNF-α-induced MCP-1 expression by azelnidipine and a protective role against atherosclerosis by suppressing MCP-1 overexpression in endothelial cells have also been reported [21]. The athero-protective effects of azelnidipine are most likely not due to its calcium channel blocking properties, but rather to its unique antioxidative characteristics. Due to its unique antioxidative property, azelnidipine may be a promising 'vascular protective long-active dihydropyridine-based calcium channel antagonist' that targets cardiovascular diseases in hypertensive patients with metabolic disease [5].

In contrast, there was no significant decrease in mean plasma MMP-9 levels after EVAR in the control group. This result may have been associated with small preoperative AAA sizes in the control group. In the present study, nearly all the excluded AAAs were small AAAs (<50 mm). It has been reported that there is a relationship between MMP-9 expression and aortic diameter [23]. The MMP-9 mRNA expression is significantly higher in medium (50- to 69 mm) AAAs than either small (30- to 49 mm) or large (>70 mm) AAAs. There was a fourfold to fivefold elevation in MMP-9 mRNA expression by aneurysms with diameters between 50 and 69 mm compared with small or large aneurysms. Besides, increased MMP-9 expression may account for the propensity of AAAs >50 mm to continue to expand, in contrast to smaller aneurysms. The preoperative average maximum diameter was under 50 mm in the control group, and over 50 mm in the azelnidipine group.

Based on these previous findings we consider that

the significant difference in preoperative MMP-9 levels between the control group and the azelnidipine group was likely due to this difference in average maximum AAA diameter. As mentioned above, there is an exponential relationship between the amount of MMP-9 produced in AAA tissue and the plasma MMP-9 levels [10]. In fact, plasma MMP-9 levels did not decrease significantly in the control group without endoleaks during the follow-up period, at least in part because preoperative plasma MMP-9 levels were low. It appears that the expression of MMP-9 in the local aneurysmal walls was not systemically reflected in the peripheral blood in the control group patients at the preoperative state.

It is interesting to note that postoperative therapy after EVAR using MMP inhibitors in the same way as in our present study has been reported recently. Hackmann et al. [24] has showed that the use of doxycycline after EVAR produced a significantly greater decrease in plasma MMP-9 levels and accelerated aneurysmal shrinkage as compared with a placebo. Doxycycline treatment significantly improved the aneurysmal shrinkage rate compared with placebo treated patients at postoperative 6 months (−13.3%±3.3% vs −3.8%±3.0%, $P<0.05$). In our present study, azelnidipine treatment resulted in about twice the shrinkage rate in maximum AAA diameters during the study period than in the control group, but the difference was not statistically significant (−14.4%±13.0% vs −7.5%±6.0%, $P= 0.094$).

Conventional open surgical repair for AAA removes all diseased aortic wall from the hemodynamic effects of the circulation, however, patients with endoleak still have an aneurysm exposed to the systemic circulation, and therefore, the biologic environment of the aneurysm would not be significantly altered. The goal of EVAR is the prevention of aneurysm-related events such as aneurysm expansion and/or late rupture, so it is expected that the excluded aneurysmal sac should shrink and disappear after EVAR. To obtain meaningful data, more patients are necessary, and long-term follow-up is needed. In conclusion, although our study involved a small sample of patients and low statistical power, it indicates that Japanese patients with no endoleaks who underwent EVAR with azelnidipine treatment experienced a significant decrease in mean plasma MMP-9 values 3 months after EVAR. Future longitudinal studies with a large number of patients are necessary to test the clinical value of MMP-9 as an independent marker of aneurysmal disease.

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Case Report

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Angiosarcoma of the Breast with Silicone Granuloma: A Case Report

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Summary: Angiosarcoma of the breast is a rare non-epithelial tumor and that accounts for less than 0.1% of primary malignancies of the breast. The disease has a relatively higher occurrence among young people, and its prognosis (3-year-survival of only 38%) is extremely poor compared to breast cancer. Here we present a case of an 87-year-old woman who had undergone bilateral breast augmentation with silicone injections in her youth. Although she became aware of a tumor in her right breast, she waited 8 years before seeking treatment. She felt the tumor growing and experienced swelling and pain, but she ended up declining therapy at that time. Two years later she was brought to our hospital by ambulance for continuous bleeding from the same tumor of the breast, which by that time was over 11 cm in diameter. We performed emergency mastectomy. The histological diagnosis was angiosarcoma of the breast with silicone granuloma.

Key words angiosarcoma of the breast, silicone granuloma, augmentation, mammoplasty

INTRODUCTION

Angiosarcoma of the breast is a rare non-epithelial tumor that accounts for less than 0.1% of primary malignancies of the breast [1]. Moreover, there has been only one report, by Cuesta et al. of the development of angiosarcoma of the breast in a patient who underwent breast augmentation with silicone implants [2]. Here we report a case of angiosarcoma with silicone granuloma, and discuss the progress of the disease in detail.

CASE REPORT

An 87-year-old woman had undergone bilateral breast augmentation with silicone injection in her youth. Although she became aware of a tumor in her right breast, she waited 8 years before seeking treatment. She underwent a medical examination at our hospital, presenting with swelling and pain in the right breast. The mass was palpable, very hard, and 7 cm in diameter. The cytological findings from fine-needle biopsy showed only blood, however, and she decided to leave the mass untreated. Two years later she came to our hospital again due to an increase in pain and swelling of the tumor, which by that time had grown to over 11 cm in diameter, along with bleeding. Although we scheduled her for hospitalization, 8 days later, she was brought to our hospital prior to that time by ambulance for continuous bleeding from the tumor.

Her medical history included left breast mastitis, a past healed fracture in the right femur, and a right cataract operation, but no history of radiation exposure. Her family history was unremarkable.

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Abbreviations: CD, cluster of differentiation; HE, hematoxylin and eosin stain; MRI, magnetic resonance imaging; US, ultrasonography.

Laboratory data were as follows: red blood cell count, $254\times10^4/\mu l$; hemoglobin, 7.6 g/dl; and C-reactive protein, 5.35 mg/dl, indicating anemia and systemic inflammation. Physical findings included hardened silicone in the bilateral breasts. The skin on her right breast was dark purple and tough, with a large tumor 11 cm in diameter with hematoma and marked protrusion (Fig. 1). Chest X-ray appeared normal, but mammography was impossible due to the tumor. Ultrasonography (US), showed a mass with solid com-

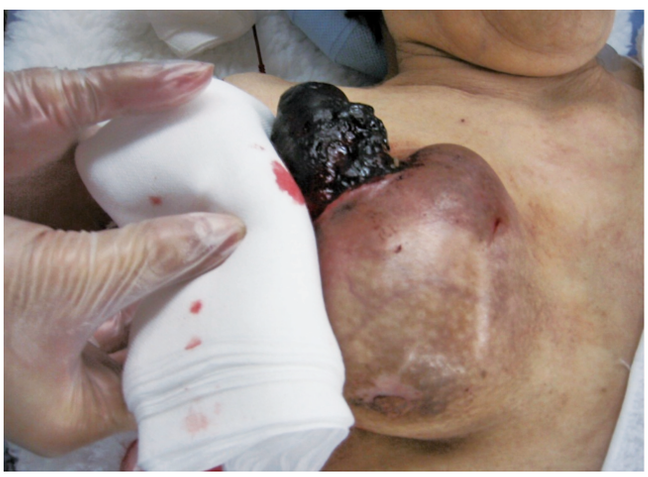

Fig. 1. The tumor had dark purple and tough skin with hematoma.

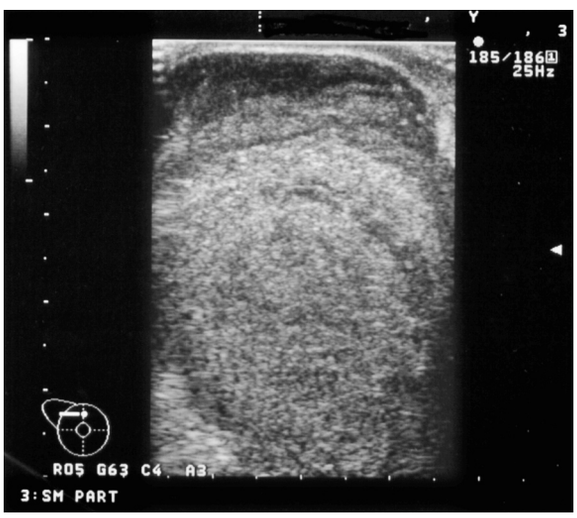

Fig. 2. US findings showed a solid mass, with low echoic area of bloody liquid.

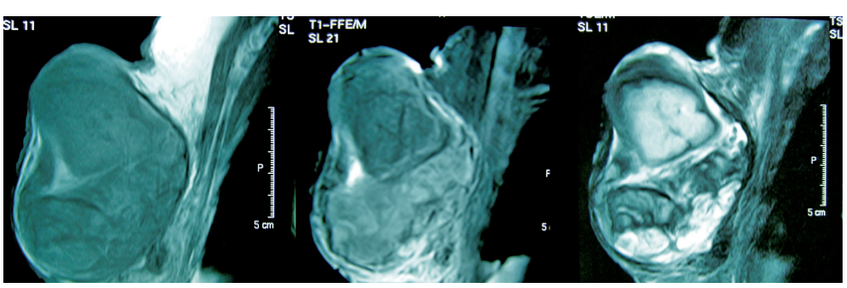

Fig. 3. MRI findings showed a mass 10.5×8×7 cm, with slight enhancement at the edge of the lower outer quadrant of the right breast in the dynamic study.

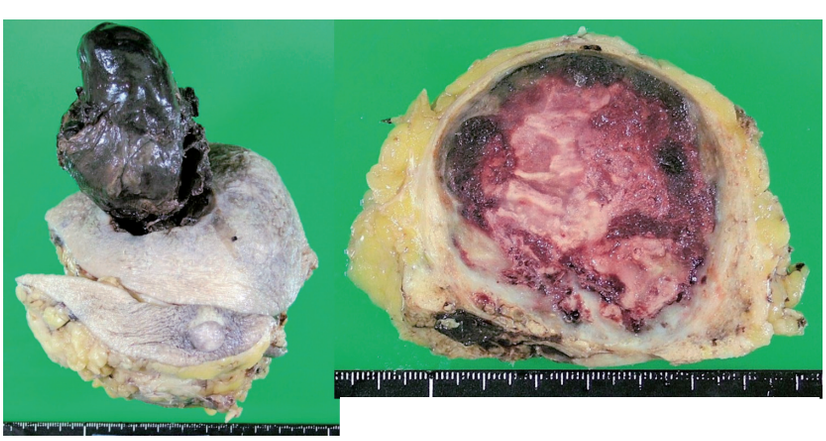

Fig. 4. Macroscopically, the tumor was 7.5×7.5 cm in size, and had a central solid pinkish area with hematoma and necrosis in parts.

ponents in her right breast. The mass included a low echoic area, which suggested that part of the tumor was filled with bloody liquid due to necrosis (Fig. 2). Magnetic resonance imaging (MRI) showed a mass 10.5×8×7 cm in size, and the edge of the lower outer quadrant of the right breast was enhanced slightly on the dynamic MRI. An area of high intensity in T2 weighted MRI suggested hematoma in the upper outer quadrant of the right breast (Fig. 3).

After a systemic examination, she was transfused for anemia. We tried to stop the bleeding by pressure with epinephrine solution gauze, but the bleeding continued. We then scheduled surgery after systemic stabilization taking into account her advanced age of 87 years. However, a projectile hemorrhage from her tumor started on the 4th day of hospitalization, and we immediately performed an emergency mastectomy. The total blood loss was 2170 cc during the 4 days just prior to and during the operation.

Macroscopically the tumor was 7.5×7.5 cm in size, with a central solid pinkish area with hematoma and partial necrosis (Fig. 4). Histologically, the central

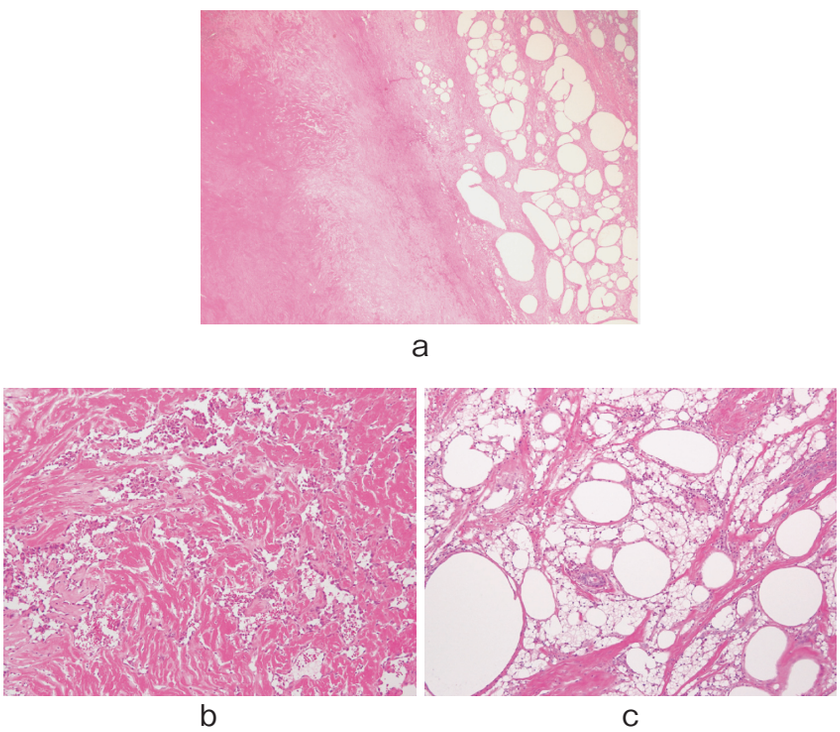

Fig. 5. Microscopic findings. (a): The central area of the tumor showed cellular degeneration (left), and the surrounding area showed silicone granuloma (right). Tumor cells were seen between regions of degeneration and granuloma (HE, low magnification). (b): The proliferation of neoplastic endothelial cells (HE, high magnification). (c): Silicone granuloma (HE, high magnification).

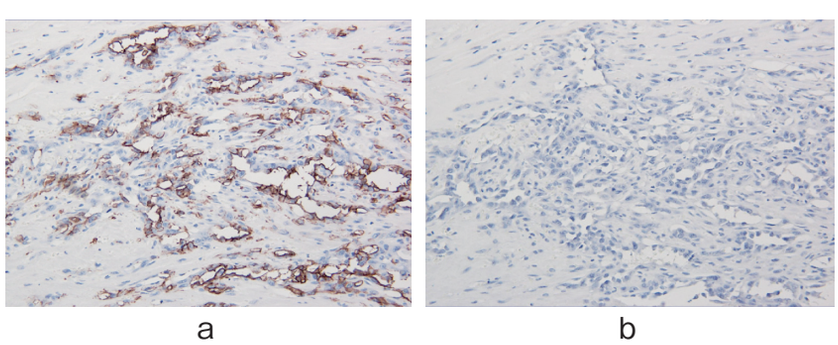

Fig. 6. On immunostaining, the neoplastic endothelial cells were positive for CD34 (Fig. 6a), and negative for CAM5.2 (Fig. 6b).

area of the tumor showed degeneration and necrosis, and the surrounding area consisted of silicone granuloma. Little viable tissue was present between the degeneration and the granuloma (Fig. 5a). The tumor cells caused expansion in papillary vascular channels, covered with neoplastic endothelial cells (Fig. 5b), and were grade Ⅱ using Rosen's 3-tier system [3]. The granuloma was seen partly within the silicone and was surrounded with hyalinization and fibrosis (Fig. 5c). Immunohistochemically, the neoplastic endothelial cells were positive for cluster of differentiation (CD) 34 and negative for cytokeratin (CAM5.2) (Fig. 6a and 6b).

Two months after the operation, she died of rapid lung metastasis, carcinomatous pleural effusion, and pneumonia.

DISCUSSION

Angiosarcoma of the breast is a rare entity that accounts for less than 0.1% of primary mammary malignancy [1]. The disease has a relatively higher occurrence among younger persons, with the average age of patients standing at 36.9 years in a range from 19 to 79 years [4].

The clinical symptoms can involve the sensation of a soft, painless mass that exhibits rapid growth. It can also involve discoloration of the skin and bleeding, as in the present case. Preoperative diagnosis of the disease is difficult, since the specimens for cytodiagnosis appear with abundant blood in the background, and angiosarcoma of the breast is often mistakenly diagnosed as benign hemangioma after fine needle aspiration biopsy. It has been reported that immunostaining is effective as an auxiliary diagnostic tool [4-6]. Due to the paucity of characteristic features in imaging tests, the average duration from the first medical examination to established diagnosis is quite long (11 months), and the average tumor is large in diameter 7.6 cm [4].

The treatment of first choice is surgical resection. Metastasis occurs mainly by hematogenous means, accordingly lymph node dissection is not necessarily required.

The prognosis is generally extremely poor, but chemotherapy (adriamycin, cisplatin, ifosfamide and paclitaxel combination [7], or weekly paclitaxel [8]) and interleukin-2 treatment has been reported to be effective [9,10]. The average survival period is 22 months with a three-year survival rate of 38% [11]. The tumor diameter is the most important prognostic factor. The five-year survival rate is 70% if the tumor diameter is less than 5 cm, but only 35% if the tumor diameter is more than 5 cm, with no significant effect from tumor grade on survival rate [12].

The relationship between breast augmentation and angiosarcoma of the breast remains unclear. The angiosarcoma and silicone granuloma in this case may have been unrelated. There has been a report, however, by Cuesta et al. on the development of angiosarcoma of the breast in a patient who underwent breast augmentation with silicone implants [2]. Multiple myeloma after silicone breast implants [13], and cutaneous T-cell lymphoma after silicone breast implants [14], has been reported in the literature. The relationship remains unclear, but T-cell-mediated autoimmune reactions may have some relationship with silicone breast implants [14]. In Japan, there have been 40 cases of breast cancer that may have been caused by breast augmentation. However, no correlation has been discovered between cancer and breast augmentation in experimental studies, and clinical case reports in the United States and Europe have also been unable to establish a correlationship [15].

In recent years, breast implants have become fashionable since a wider variety of breast augmentation methods have become available, including silicone infusion, silicone bag implant, normal saline solution bag implant, and hyaluronic acid infusion. In part the increase in breast implants may be attributed to changes in women's values and concepts of beauty. However, in many cases, diagnosis of neoplasm of the breast after breast augmentation is difficult. Therefore, we ought to take into consideration the risks from as-yet-unknown foreign-body reactions and complicating factors.

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