SPANDIDOS PUBLICATIONS

Combination therapy of tyrosine kinase receptor inhibitor TSU-68 (SU6668) and paclitaxel inhibits subcutaneous xenografts of endometrial cancer

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Abstract. TSU-68 is a small-molecular-weight synthetic inhibitor of the tyrosine kinase receptors Flk-1/KDR, PDGFRß and FGFR1, which are involved in angiogenesis. Using a mouse model in which endometrial cancer was subcutaneously implanted, we investigated the effects of TSU-68 alone or in combination with paclitaxel. We subcutaneously implanted a cell strain of endometrial cancer, HEC1A, into BALB/c nude mice. TSU-68 was orally administered every day, while paclitaxel was intraperitoneally injected once a week, and the rates of subcutaneous tumor proliferation were compared. In a group treated with high-dose (200 mg/kg/day) TSU-68 alone, subcutaneous tumor proliferation was significantly inhibited in comparison with a vehicle-treated control group ($p<0.05$). In groups treated with low-dose TSU-68 or paclitaxel alone (100 and 10 mg/kg/day, respectively), tumor proliferation was not significantly inhibited. In a low-dose combination therapy group (100 mg/kg/day of TSU-68 + 10 mg/kg/day of paclitaxel), tumor proliferation was significantly inhibited in comparison with the control and low-dose TSU-68 or paclitaxel therapy groups ($p<0.01$). High-dose monotherapy with TSU-68 inhibited the proliferation of the subcutaneously implanted tumor. Furthermore, a combination of TSU-68 and paclitaxel at a low dose, one at which respective monotherapy was not effective, inhibited tumor proliferation. Combination therapy with the two agents may therefore be useful for treating endometrial cancer.

Introduction

In the United States, endometrial cancer is the fourth most common disease among malignant tumors in women. The annual number of patients newly developing this cancer is approximately 40,000 (1). In most patients, endometrial cancer is detected at an early stage, when the cancer is localized in the uterus. As, in such cases, radical surgery is possible, the 5-year survival rate exceeds 80% (2). However, in advanced endometrial cancer patients with a FIGO stage of III/IV, which reflects extrauterine invasion, the 5-year survival rate remains low (0-40%) (2,3). In such patients, radiotherapy and combination chemotherapy have recently been applied in addition to surgery. However, the results of these are not satisfactory, and consequently the outcome of endometrial cancer treatment has not improved over the past 30 years (1). A new therapeutic strategy for the treatment of advanced endometrial cancer should therefore be established.

The proliferation and invasion of malignant tumors depend on angiogenesis (4). A recent study suggested the clinical usefulness of angiogenesis-inhibiting therapy for cancer, increasing its importance (5). Factors involved in tumor angiogenesis include vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) (6-9). In particular, VEGF plays a primary role (6). In patients with endometrial cancer, there was also a correlation between VEGF and angiogenesis (10), and VEGF has been reported to be a factor influencing the prognosis of endometrial cancer patients (10-12). This suggests the usefulness of angiogenesis-inhibiting therapy for endometrial cancer.

TSU-68, {SU6668:(Z)-5-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-ropanoic acid}, is a small-molecular-weight synthetic kinase inhibitor of fetal liver kinase-1/kinase insert domain-containing receptor (Flk-1/KDR), PDGF receptor ß (PDGFRß), and FGF receptor 1 (FGFR1), for VEGF, PDGF and bFGF, respectively (13,14). It binds to the adenosine triphosphate-binding pocket of these three tyrosine kinase receptors and competitively inhibits their phosphorylation, thereby exhibiting inhibitory effects on vascular endothelial cell growth (13,14). Several studies using various models in which solid cancer was subcutaneously implanted have confirmed that TSU-68 inhibits tumor angiogenesis and growth. Furthermore, this agent has been reported to induce apoptosis in vascular endothelial and tumor cells (14,15).

In this study, we examined whether TSU-68 inhibits tumor proliferation in a subcutaneously endometrial cancer-implanted

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model for the purpose of its clinical application in patients with endometrial cancer. In addition, we investigated the effects of combination therapy with TSU-68 and paclitaxel, a conventional anticancer agent that has been reported to be effective for advanced endometrial cancer (16,17).

Materials and methods

Cell line. A human endometrial endometrioid adenocarcinoma cell line, HEC-1A, was provided by Dr Kuramoto (Kitasato University, Japan).

Animals. We purchased 6-week-old female BALB/c nude mice from Clea Laboratories (Tokyo, Japan). The mice were acclimated under a pathogen-free environment. All animal experiments were performed according to the guidelines established by Jichi Medical University.

Drug administration. TSU-68 (SU6668) was provided by Taiho Pharmaceutical Co., Ltd. (Tokyo, Japan). We subcutaneously inoculated $6x10^6$ cells/body of HEC-1A into BALB/c nude mice. Drug administration was initiated two weeks after inoculation. TSU-68 was dissolved in a carboxymethylcellulose vehicle, and orally administered 6 times a week. Paclitaxel, supplied by Bristol-Myers Squibb Co. (Tokyo, Japan), was dissolved in 50% cremophor EL and 50% dehydrated alcohol, then diluted with physiological saline. The solution was intraperitoneally injected once a week.

For monotherapy, high-dose TSU-68 (200 mg/kg/day) was orally administered every day. In the control group, a vehicle was administered.

For combination therapy, the mice were divided into 5 groups: low-dose TSU-68 monotherapy (100 mg/kg/day), low-/high-dose paclitaxel monotherapy (10 and 20 mg/kg/day, respectively), low-dose combination therapy (100 mg/kg/day of TSU-68 and 10 mg/kg/day of paclitaxel), and control (vehicle) groups.

Evaluation of treatment response. Subcutaneous tumor volume was calculated using the following formula: maximum diameter x minimum diameter^2 x 1/2. The rate of subcutaneous tumor proliferation was calculated using the following formula: tumor volume on the day of measurement/tumor volume at the start of drug therapy.

Statistical analysis. Significance was tested using the unpaired Student's t-test. $P<0.05$ was regarded as significant.

Results

Inhibitory effects of TSU-68 on subcutaneous tumor proliferation. In the high-dose TSU-68 monotherapy group, the rate of proliferation was significantly lower than in the control group ($p<0.05$, Fig. 1), and the oral administration of TSU-68 alone inhibited endometrial tumor proliferation.

Inhibitory effects of low-dose combination therapy with TSU-68 and paclitaxel on subcutaneous tumor proliferation. There were no significant differences in the rate of proliferation between the low-dose TSU-68/paclitaxel monotherapy and control groups. In the low-dose combination therapy group, the rate of proliferation was significantly lower than in the control and respective low-dose monotherapy groups ($p<0.01$). In the high-dose paclitaxel monotherapy group, tumor proliferation was significantly inhibited in comparison with the control group ($p<0.01$). However, there was no significant difference between this and low-dose paclitaxel monotherapy groups.

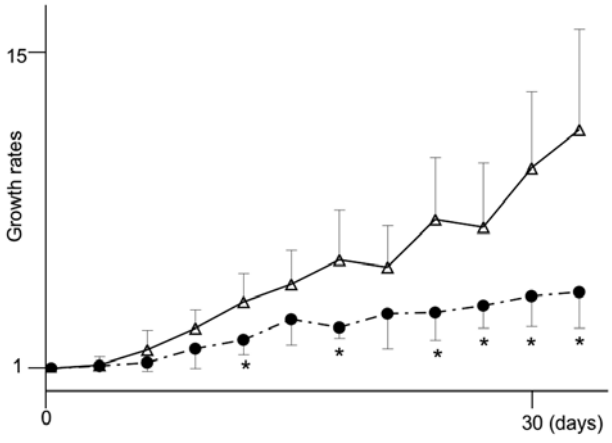

Figure 1. Effects of TSU-68 monotherapy on the subcutaneous tumor. From two weeks after the subcutaneous inoculation of the HEC1A cell strain of endometrial cancer ($6x10^6$), TSU-68 at 200 mg/kg/day (black circle, n=6) or a vehicle (white triangle, n=6) were orally administered every day. The rate of tumor proliferation after the start of drug administration, which was significantly lower in the TSU-68-treated group than in the control (vehicle) group (*$p<0.05$), is shown.

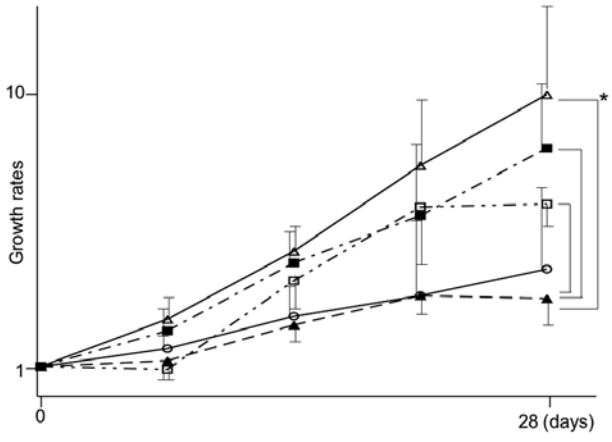

Figure 2. Effects of combination therapy with TSU-68 and paclitaxel on the subcutaneous tumor. From 2 weeks after the subcutaneous inoculation of HEC1A, TSU-68 was orally administered every day, and paclitaxel was intraperitoneally injected once a week. Based on treatment methods, animals were divided into 5 groups: a control (vehicle) group (white triangle, n=12), a group treated with 100 mg/kg/day of TSU-68 (black square, n=4), a group treated with 10 mg/kg/day of paclitaxel (white square, n=4), a group treated with 20 mg/kg/day of paclitaxel (white circle, n=8), and a group treated with the combination of TSU-68 at 100 mg/kg/day and paclitaxel at 10 mg/kg/day (black triangle, n=4). Neither single low-dose TSU-68 nor paclitaxel therapies significantly inhibited tumor proliferation in comparison with the control group. Low-dose combination therapy significantly inhibited tumor proliferation in comparison with vehicle treatment and respective single low-dose therapy (*$p<0.01$).

In this study using a mouse model, the oral administration of TSU-68 alone inhibited the proliferation of subcutaneously implanted endometrial cancer. In addition, combination therapy with TSU-68 and paclitaxel at a low dose, one at which respective monotherapy was not effective, inhibited tumor proliferation.

Currently, some molecule-targeting agents are applied in clinical practice to treat various malignant tumors. In Europe and the United States, tyrosine kinase inhibitors such as imatinib mesilate, gefitinib, erlotinib, the Raf kinase inhibitor sorafenib, the anti-HER2 antibody trastuzumab and the anti-human VEGF monoclonal antibody bevasizumab, have been approved and applied in clinical practice to treat non-small cell lung, kidney, breast and metastatic rectal cancer, respectively (18,19).

Few studies have reported molecule-targeting therapy for endometrial cancer. Kamat *et al* reported that expression of the most important angiogenic factor, VEGF, was marked in more than 50% of patients with endometrial cancer, suggesting that high-level VEGF expression is an independent prognostic factor (20). Based on their findings, we designed the current study, considering that VEGF-targeting therapy may be effective for endometrial cancer.

TSU-68 is a small-molecular-weight synthetic kinase inhibitor of the receptors Flk-1/KDR, PDGFRß and FGFR1 (13,14). We previously reported the inhibitory and survival-prolonging effects of orally-administered TSU-68 on tumor angiogenesis and peritoneal dissemination using a peritoneally-disseminated ovarian cancer model in mice (21). The current study, also using a mouse model, demonstrated the antitumor effects of TSU-68 on subcutaneously implanted endometrial cancer, suggesting the usefulness of molecule-targeting therapy with TSU-68 for endometrial cancer. As the HEC1A endometrial cancer cell strain employed in this study secreted a large amount of VEGF (data not shown), the inhibitory effects of TSU-68 on tumor proliferation, as observed in this study, may have been mediated by VEGF.

Phase I clinical studies of TSU-68 in patients with solid tumors have been conducted (22,23). Dose-limiting toxicities included abdominal pain, anorexia, nausea/vomiting, pericardial effusion and thrombocytopenia (22,23). According to Kuenen *et al*, the maximum tolerated dose was 100 mg/m^2 t.i.d., and no objective response was achieved in any patient. As a sufficient plasma level is not obtained at this dose, monotherapy with TSU-68 may not be effective (22).

Currently, the tumor-regressing effects of molecule-targeting therapy alone are limited. Therefore, in clinical practice, it is combined with conventional anticancer drugs in many patients (24-28). As a representative regimen, combination therapy with bevasizumab, fluorouracil, and leucovor for colorectal cancer is known (27,28). TSU-68 may also be clinically useful when combined with anticancer drugs.

Garofalo *et al* conducted a basic study using a peritoneally-disseminated ovarian cancer mouse model to evaluate the effects of combination therapy with TSU-68 and paclitaxel (29). The combination of TSU-68 at 200 mg/kg/day orally administered every day and paclitaxel at 20 mg/kg/day intravenously injected once a week achieved synergistic inhibitory effects on tumor proliferation/ascites production and survival-prolonging effects in comparison with monotherapy (29). Naumova *et al* performed a basic study to clarify the mechanism involved in the efficacy of combination therapy with TSU-68 and paclitaxel (30). The combination more potently inhibited the proliferation of human umbilical vein endothelial cells (HUVECs) and human microvascular endothelial cells, and induced HUVEC apoptosis compared to monotherapy (30).

Paclitaxel causes the stabilization/hyperplasia of microtubules by inhibiting their depolymerization, and exhibits anticancer actions by suppressing cell division (31). The agent also inhibits endothelial cell proliferation at a low concentration (32,33), acting not only on tumors but also on blood vessels, which may contribute to the effects of combination therapy with TSU-68.

In a clinical study, the plasma levels of TSU-68 administered at a tolerated dose were low, and TSU-68 alone may not be effective. In this basic study, the combination of TSU-68 and paclitaxel at a low dose, at which respective monotherapy does not inhibit tumor proliferation, exhibited inhibitory effects on tumor proliferation. Combination therapy with these two agents may be a new strategy for the treatment of endometrial cancer.

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