Differential Effect of Intraperitoneal Albendazole and Paclitaxel on Ascites Formation and Expression of Vascular Endothelial Growth Factor in Ovarian Cancer Cell Bearing Athymic Nude Mice

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Differential Effect of Intraperitoneal Albendazole and Paclitaxel on Ascites Formation and Expression of Vascular Endothelial Growth Factor in Ovarian Cancer Cell Bearing Athymic Nude Mice

Directed by Professor Young-Tae Kim

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This certifies that the Doctoral Dissertation of Eun-Kyoung Choi is approved

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Eun Kyoung Choi

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<ABSTRACT>

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Vascular endothelial growth factor (VEGF) is supposed to play a pivotal role in tumor metastasis and ascites formation in epithelial ovarian cancer and combined use of VEGF inhibitor and cytotoxic chemotherapeutic agents in treatment of ovarian cancer patients showed some synergistic effect. Recently, it was claimed that albendazole showed suppressive effects on VEGF expression and ascites formation on some kinds of cancer cell lines in *in vivo* and *in vitro*. The present study was aimed to evaluate the effect of weekly intraperitoneal albendazole on tumor growth, ascites formation and VEGF mRNAs expression and possible synergistic effect of paclitaxel in the OVCAR-3 ovarian cancer xenograft model.

Four groups of female nude mice with peritoneal carcinomatosis of OVCAR-3 human ovarian cancer cell line were treated with weekly intraperitoneal injection of one of the following for 4 wk : albendazole

alone (450 mg/kg/wk), paclitaxel alone (30 mg/kg/wk), albendazole plus paclitaxel (450 mg/kg/wk of albendazole plus 30 mg/kg/wk paclitaxel), and normal saline only (for control group). After completion of scheduled therapy, mice were euthanized with a lethal dose of CO_2 and tumor burden, ascites volume, VEGF levels in ascites fluid, and VEGF mRNAs expression were measured and compared with each treatment group. The expression of VEGF mRNA was also assessed in *in vitro* cultured OVCAR-3 cells with various concentrations of albendazole, paclitaxel and combination of albendazole and paclitaxel.

A significant reduction in the ascites volume and in ascitic fluid VEGF levels was noted in all three treatment groups, irrespective of drug regimen. However, complete tumor mass suppression was more prominent in the paclitaxel-treated group, and VEGF mRNA expression was more strongly inhibited in the albendazole-treated group. Interestingly, VEGF mRNAs expression was significantly up-regulated in the paclitaxel-treated group in both *in vivo* and *in vitro*. We failed to show any synergistic effect in combined administration of albendazole and paclitaxel.

Our results showed that weekly intraperitoneal administration of albendazole and paclitaxel effectively suppressed ascites formation and decreased ascitic fluid VEGF levels in the OVCAR-3 cell bearing athymic nude mice model, and there was no demonstrable synergistic effect in combination of two drugs. In contrast with the suppression of VEGF mRNAs expression observed in the albendazole treated group, VEGF mRNAs were paradoxically up-regulated in the paclitaxel treated group. Hence, we could speculate the mechanism of suppression

of ascites formation of albendazole and paclitaxel would be different : while albendazole suppressed ascites formation by inhibition of VEGF mRNA expression and paclitaxel exerted its antiascitic effects by direct cytotoxicity against VEGF-secreting tumor cells.

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key words : albendazole, paclitaxel, ascites,
vascular endothelial growth factor , ovary cancer

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I. INTRODUCTION

Epithelial ovarian cancer is the most lethal gynecologic malignancy.[1] About two thirds of ovarian cancer patients already have advanced disease when diagnosed, and rapidly accumulating ascitic fluid and large volume of solid intraperitoneal tumor mass are associated with poor prognosis[2] but malignant progression of this disease is often predominantly confined to the peritoneal cavity.[3] This characteristic tumor behavior of ovarian cancer is associated with increased tumor vessel formation and increased vascular permeability and vascular endothelial growth factor (VEGF) plays a pivotal role both in tumor growth, metastasis and often massive ascites formation both in primary and recurrent ovarian cancer.[4] Thus, VEGF inhibitors could be an attractive candidate in treatment of ovarian cancer and showed actual antitumor activity by decreased visible ascites formation and reduced tumor burden in a xenograft models of advanced human ovarian cancer in some published series.[5-8]

Considering pathophysiology of ovarian cancer, we can expect that combination of VEGF inhibitor and cytotoxic chemotherapeutic agents could

have some synergistic effect for treatment and several clinical studies have been conducted.[9-12] However, the therapeutic efficacy of VEGF inhibitor seems to be somewhat limited. VEGF assists tumor growth and metastasis by setting its own vasculature to supply the oxygen and nutrients and has no direct mitogenic or growth promoting effect. Consequently, VEGF inhibitor might have a greater effect in inhibition of tumor metastasis or recurrence than reduction of tumor mass that is already settled down. In other words, VEGF inhibitor is more suitable for maintenance of remission than remission induction in clinical setting. Currently available VEGF inhibitors, such as anti-VEGF antibody or tyrosine kinase inhibitors, are not appropriate for maintenance therapy because of its high expense and systemic adverse side effects.

Albendazole, a benzimidazole carbamate antihelmintic drug, has been in clinical use for almost three decades. As an oral antihelmintic, its efficacy and safety has been well established.[13] The primary mode of action of albendazole in susceptible parasites has been described as binding to ß-tubulin and leading to inhibition of microtubule polymerization[14-5] and shares similar mechanism of action with some anticancer agents. Morris et al. paid attention to this microtubule polymerization inhibitory effect of albendazole and made a pilot study where some patients with colorectal cancer and liver metastases had shown a decline or stabilization of tumor marker (carcinoembryonic antigen) when treated with oral albendazole and this antitumor effect is more closely related with inhibition of neovascularization than inhibition of microtubule polymerization. [16] This report suggested albendazole might be able to suppress the angiogenesis and expression of VEGF. After this, albendazole and its close analogue mebendazole has demonstrated a considerable effect on tumor growth and

tumor vessel formation in some kinds of cancer cell lines.[17-20] Poor water solubility and extensive first-pass metabolism make its clinical application as an oral anticancer agent difficult but these pharmacokinetic characteristics are even more attractive for intraperitoneal application to the treatment of peritoneal carcinomatosis.

If periodic injections of albendazole could suppress tumor growth, distant metastasis and ascites formation in ovarian cancer patient by itself or in company with other cytotoxic agent, it would be a great help for treatment of ovarian cancer. Albendazole is a relatively cheap and safe drug with little systemic toxicity. Even though albendazole could not eliminate large tumor mass like other VEGF inhibitors, it might have synergistic effect to cytotoxic chemotherapy or inhibitory effect for growth of small metastatic nodule by its antiangiogenic effect.

We carried out this study to assess effect of intraperitoneal albendazole on inhibiting ascites formation and expression of VEGF in ovarian cancer xenograft model and investigate the possible synergistic effect with paclitaxel, the typical anticancer chemotherapeutic agent used in treatment of ovarian cancer.

II. MATERIALS AND METHODS

1. Chemicals

Paclitaxel (Hanmi Pharm., Seoul, Korea) and albendazole (Daewoong Pharm., Seoul, Korea) were used for this experiment. For the *in vitro* and *in vivo* assay, albendazole was suspended in 0.5% carboxymethyl cellulose.

2. Cell line and culture

We selected the OVCAR-3 cell line for our experiments because in this cell line, tumor growth is more restricted to easily identifiable surfaces and ascites develops earlier in the course of disease progression as compared to the SKOV-3 cell line; therefore, we could anticipate more accurate quantification of the treatment effects on tumor growth and ascites formation.

Cells of the human ovarian cancer cell line OVCAR-3 (Korean Cell Line Bank, Seoul, Korea) were seeded into a culture plate (concentration, 2×10^4 cells/well) containing RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum and 50 units/ml of penicillin, 50 units/ml of streptomycin, and 25 units/ml amphotericin B at 37°C in humidified incubators containing 5% CO_2 incubator. Cells were grown to adequate confluence and harvested by trypsinization with 0.25 mg/ml trypsin/EDTA and suspended in the medium. All cell culture reagents we used were purchased from Gibco, Invitrogen (Carlsbad, CA, USA).

3. Culture and drug treatment in *in vitro*

The precultured OVCAR-3 cells were shifted onto a 96-well plate at a concentration of 2×10^3 cells/well. After incubation for 24 hr, the culture

medium was removed. The cells that remained were washed thrice with phosphate buffer and incubated with a serum-containing growth medium and varying concentrations of the one of the followings : albendazole (2, 20, 200 nM), paclitaxel (0.2, 1, 20 μM), albendazole plus paclitaxel (albendazole 2, 20, 200 nM plus paclitaxel 20 μM)

4. Establishment of ovarian cancer xenograft model

For all experiments, 5- to 6-week-old female nude athymic BALB/c *nu/nu* mice (Central Lab. Animal Inc., Seoul, Korea) were used. They were acclimatized for 1 wk, maintained under specific pathogen-free conditions, and were fed autoclaved pellets and water *ad libitum*. The general health status of the animals was monitored daily. All animal experiments were approved and supervised by the institutional ethical committee.

The precultured OVCAR-3 cells were washed with sterile saline three times , centrifuged at 12,000 rpm at 4°C for 5 min to collect floating tumor cells, and then immediately inoculated into the test animals. Each animal was injected intraperitoneally with 1×10^7 viable tumor cells suspended in 1.0 ml of RPMI 1640 medium.

5. Drug treatment and sample collection

After 3 wk, development of ascites was noted and confirmed by comparison with the normal controls. First, the peritoneal cavity was washed with 2 ml of sterile normal saline. The peritoneal contents were mixed by kneading and then completely aspirated. Further, mice were randomly distributed into 1 of the 3 treatment groups (albendazole, paclitaxel, and combined albendazole plus paclitaxel) or into the experimental control group (n = 10 per group). Drug treatment was initiated immediately after ascitic fluid aspiration and

body weight measurement. Mice were administered albendazole (450 mg/kg), paclitaxel (30 mg/kg), or combined albendazole plus paclitaxel (450 mg/kg of albendazole plus 30 mg/kg paclitaxel) once in a week for 4 wk, according to the group to which they belonged. Although the dose was chosen based on certain references[5, 21], the injection schedule was changed from a thrice-a-week regimen to a once-a-week regimen because both albendazole and paclitaxel exert their actions in a dose-dependent manner, and we attempted to mimic the clinical chemotherapy schedule as much as possible. At the end of the treatment period, mice were euthanized with a lethal dose of CO_2. Although the intended duration of treatment was 4 wk, the animals were euthanized if either their abdominal circumference reached 9.5 cm or if they were expected to die within a short period of time—a requirement of the institutional animal ethics committee. Following euthanasia, 2 ml of normal saline was injected intraperitoneally, and the peritoneal cavity was completely washed and its contents, aspirated. The actual ascitic fluid volume collected after each aspiration was calculated by subtracting 2 ml from the total volume collected. All visible intraperitoneal tumor masses were carefully collected through laparotomy and weight of total mass was measured. Obtained ascites and dissected tumors from the peritoneal cavity were all stored at – 80°C for subsequent analysis.

6. Quantitative assay of ascitic fluid VEGF levels

Before attempting to measure the precise levels of VEGF in the ascitic fluid or plasma, Western blot analysis was conducted on cell-free ascitic fluid collected from the peritoneal cavity of the mice in order to verify the presence of the VEGF protein. Following this, ascitic fluid VEGF levels were

measured by an ELISA that detects soluble $VEGF_{165}$ (Quantikine; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

7. Determination of VEGF mRNA expression in *in vivo* and *in vitro*

After harvested, washed with sterile saline and centrifuged at 1200 rpm and 4°C for 5 min , 2 x 10^6 tumor cells collected from *in vitro* assay and tumor mass of mouse were examined for the expression of various isoforms (121, 165, 189) of VEGF mRNA by reverse transcription-PCR. Total RNA was isolated from the cells using the Total RNA extraction kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. Primers for the amplification of VEGF were constructed based on the following sequence: VEGF sense, 5'-CAC ATA GGA GAG ATG AGC TTC-3; VEGF antisense, 5'-CCG CCT CGG CTT GTC ACA T-3'. These primers amplify the following products: 100 bp for $VEGF_{121}$, 230 bp for $VEGF_{165}$, 300 bp for $VEGF_{189}$. The ß-actin gene was used as an internal control (331 bp; ß-actin sense, 5'-AGG CCA ACC GCG AGA AGA TGA CC-3' ; ß-actin antisense, 5'-GAA GTC CAG GGC GAC GTA GCA C-3'); 1 µg of total RNA was used to amplify VEGF/ß-actin using the SuperScript One Step reverse transcription-PCR with Platinum Taq (Invitrogen, Carlsbad, CA, USA). The amplification was carried out in a Palm Cycler after an initial cDNA synthesis in 5 min at 65°C, 50 min at 50°C, 5 min at 85°C and 5 min at 94°C for denaturation. This was followed by 35 cycles (denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min, and primer extension at 72°C for 45 s for VEGF, denaturation at 94°C for 30 s, primer annealing at 60°C for 30 s, and primer extension at 72°C for 30 s for ß-actin) and a final elongation of 72°C for 7 min. The reverse transcription-polymerase chain reaction (RT-PCR)

products were visualized by electrophoresis (25 min at 120 V) on 2% agarose gel in 1 x TAE buffer containing ethidium bromide. To quantify the size of the products, a 100-bp DNA ladder was run with the samples.

8. Statistics

Data were analyzed using parametric and nonparametric statistics, SPSS 12.0 (Chicago, IL, USA). Descriptive statistics were used for demographic baseline data and are summarized as means and standard deviations. Continuous variables were examined for a normal distribution (Kolmogorov-Smirnov test) before adopting parametric statistics. Differences between continuous variables were evaluated by One-way ANOVA test for normally distributed variables and by the Kruskal-Wallis test for variables that were not normally distributed. Categorical variables were evaluated with the χ^2-test. Differences were considered significant when their probability of being due to random chance was below 5% ($P < 0.05$).

III. RESULTS

1. Effects of intraperitoneal albendazole and paclitaxel on tumor growth in mice inoculated with OVCAR-3 cells

Grossly visible tumor masses on the peritoneal surface were detected in 80% of the mice in the control and albendazole-treated groups, 30% of the mice in the paclitaxel-treated group, and 40% of the mice in the combined albendazole plus paclitaxel-treated group.

The number of mice developing visible tumor masses differed significantly between the non-paclitaxel- and paclitaxel-treated groups ($p < 0.05$). However, since these tumors were considerably small (<5 mm) and were distributed in a miliary pattern along the entire peritoneal surface (Fig. 1), we were unable to collect and measure the precise weight of the tumor masses in each treatment group. .

Fig. 1. Tumor mass formation following intraperitoneal OVCAR-3 inoculation to the nude mice. After inoculation of the OVCAR-3 cells into the peritoneal cavity, milliary tumor nodules were produced along the entire peritoneal surface including the bowel serosa, omentum and ovarian surface (white arrow). Although the number of mice developing visible tumor masses differed significantly between the non-paclitaxel- and paclitaxel-treated groups, intraperitoneal tumor growth patterns of 4 treatment groups did not show any differences.

2. Inhibition of ascites formation with intraperitoneal administration of albendazole and paclitaxel in OVCAR-3 inoculated nude mice

Following initial aspiration, the control mice continuously produced ascitic fluid at a high rate. In contrast, mice treated with any treatment showed remarkable decrease in ascites production irrespective of drug regimen (Fig.2)

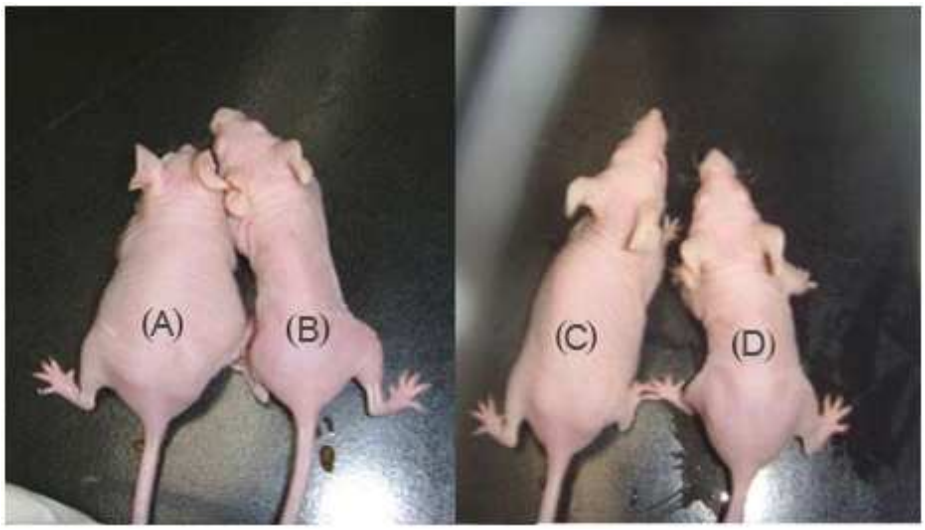

Fig. 2. Inhibition of ascites formation by intraperitoneal administration of albendazole and paclitaxel in mice inoculated with OVCAR-3 cells. While nude mice inoculated with only OVCAR-3 cells (A) developed overt ascites, there were no macroscopic signs of ascites formation in the albendazole–treated (B), paclitaxel–treated (C), and combined albendazole plus paclitaxel-treated (D) mice.

The mean cumulative ascites volume in the control group was 5.90 ± 1.75 ml and those in the treatment groups were 2.47 ± 0.93 ml (albendazole-treated group), 2.65 ± 1.80 ml (paclitaxel-treated group), and 2.88 ± 1.13 ml

(combined albendazole plus paclitaxel-treated group), respectively. Albendazole, paclitaxel, and combined albendazole plus paclitaxel treatment brought about a statistically significant decrease in the ascites volume ($p < 0.05$) ; however, there was no statistically significant difference in the therapeutic effect among the 3 treatment groups(Fig.3).

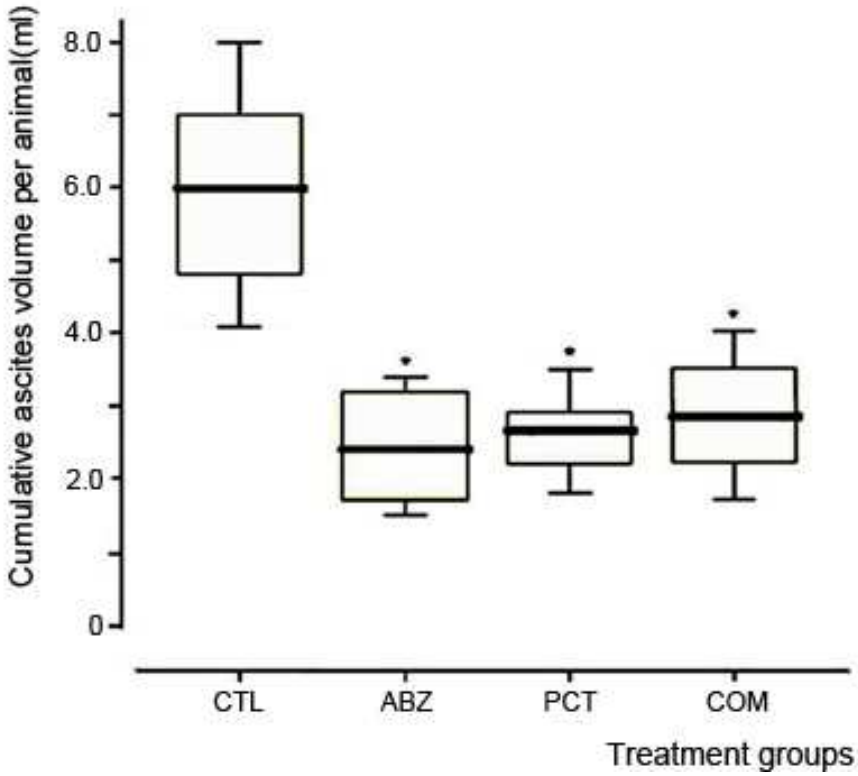

Fig. 3. The mean cumulative ascites volume in the OVCAR-3-inoculated nude mice with no treatment (CTL) and those treated with albendazole (ABZ), paclitaxel (PCT), and combined albendazole plus paclitaxel (COM). Albendazole, paclitaxel, and combined albendazole plus paclitaxel treatment brought about a statistically significant decrease in the ascites volume ($p < 0.05$) ; however, there was no statistically significant difference in the therapeutic effect among the 3 treatment groups Columns: mean; bars: SD; *: $p < 0.05$ versus control.

3. Suppression of ascites fluid VEGF levels by intraperitoneal albendazole and paclitaxel in OVCAR-3 inoculated nude mice

We determined the ascitic fluid VEGF levels by using the standard ELISA kit measures $VEGF_{165}$, the predominant and probably the most important VEGF isoform[22]. The results showed a statistically significant reduction in the ascitic fluid VEGF levels (Fig. 4) in all the treatment groups (170.83 ± 97.69 pg/ml, 229.16 ± 148.67 pg/ml, and 267.00 ± 153.93 pg/ml in the albendazole-, paclitaxel-, and combined albendazole plus paclitaxel-treated groups, respectively) as compared to the control group (1625.00 ± 1352.31 pg/ml) irrespective of the drugs administered ($p < 0.05$). However, the difference between the 3 treatment groups was not statistically significant.

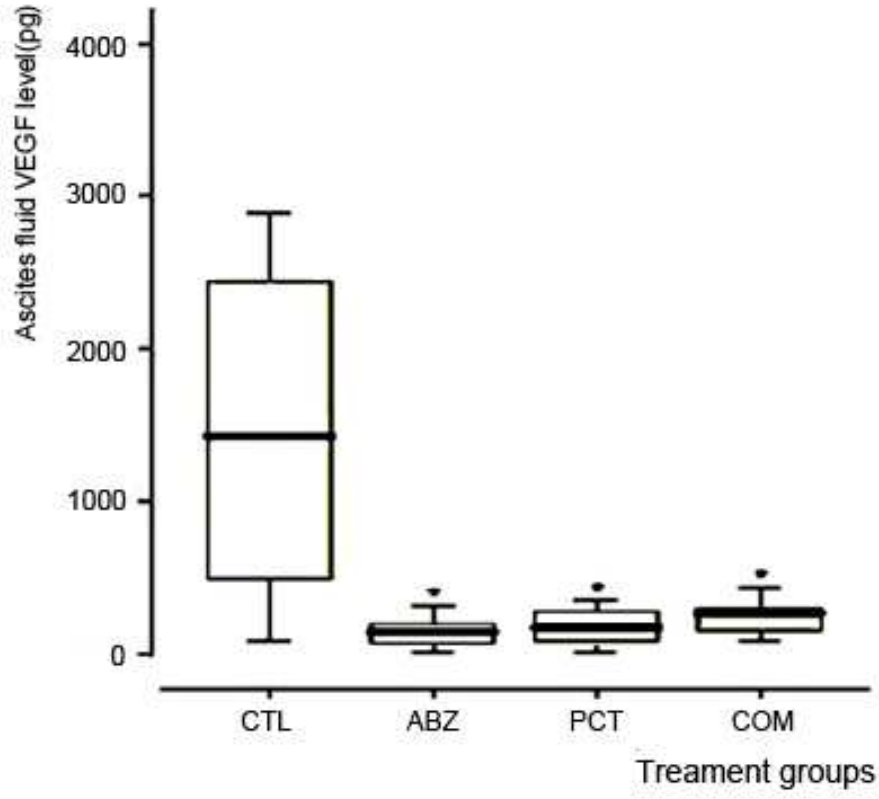

Fig. 4. The ascitic fluid VEGF levels in the OVCAR-3-inoculated nude mice with no treatment (CTL) and those treated with albendazole (ABZ), paclitaxel (PCT), and combined albendazole plus paclitaxel (COM). The re showed a statistically significant reduction in the ascitic fluid VEGF levels in all 3 treatment as compared to the control group, irrespective of the drugs administered ($p < 0.05$). However, the difference between the 3 treatment groups was not statistically significant. Columns: mean; bars: SD; *: $p < 0.05$ versus control.

4. Differential effects of albendazole and paclitaxel on VEGF mRNAs expression in *in vivo* and *in vitro*

Using reverse transcription-PCR, VEGF mRNA was extracted from the tumor cells from the tumor mass and the ascitic fluid of experimental animals and analyzed. By this method, 3 different bands corresponding to $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$ were detected (Fig. 5).

The reduction in the density of the VEGF bands from the albendazole-treated samples was statistically significant ($p < 0.05$), indicating down-regulation of VEGF mRNA in the OVCAR-3 cells from the albendazole-treated mice. However, in case of the paclitaxel-treated mice, a small but evident up-regulation of VEGF mRNA expression ($p < 0.05$) was noted and VEGF mRNA up-regulation by paclitaxel and down-regulation by albendazole appears to be neutralized when a combination of both drugs was used. As a result, there was no synergistic effect of albendazole and paclitaxel that we initially anticipated and moreover, the suppressive effect of albendazole on VEGF mRNAs expression appeared to be somewhat diminished by adding paclitaxel ; granting it failed to show a statistical significance.

This differential effect of albendazole and paclitaxel on VEGF mRNA expression was also demonstrated in *in vitro* experiments (Fig. 6). The VEGF mRNAs expression from OVCAR-3 cells incubated with albendazole were diminished while those from the cells incubated with paclitaxel was increased in the dose dependent manner.

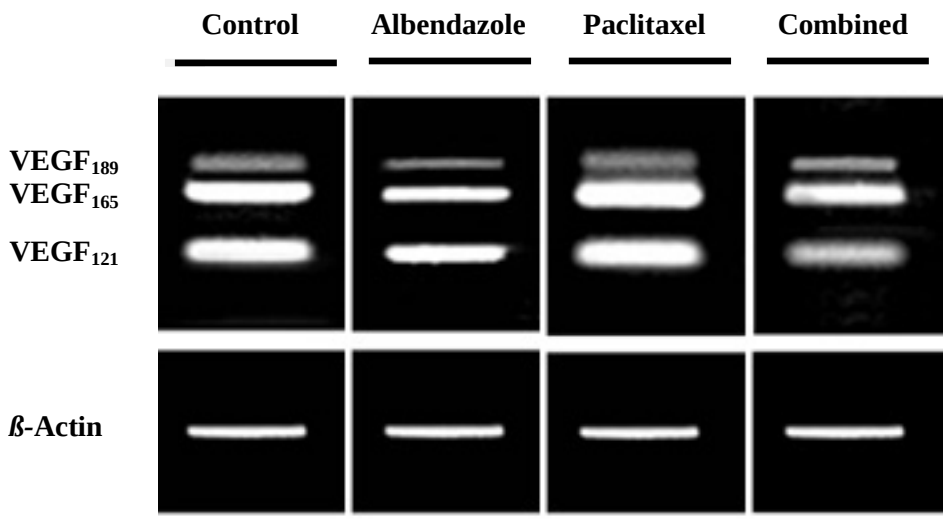

Fig. 5. Expressions of $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$ mRNA in the cells obtained from the tumor masses and ascites of OVCAR-3-inoculated nude mice with no treatment(control) and those treated with albendazole, paclitaxel, and combined albendazole plus paclitaxel by RT-PCR. 3 different bands corresponding to $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$ were detected . *ß*-Actin gene was used as internal control. Products were visualized by electrophoresis on 2% agarose gel in 1× TAE buffer containing ethidium bromide. Expression of VEGF mRNAs was down-regulated by albendazole and up-regulated by paclitaxel. The effect of albendazole on VEGF mRNAs expression appeared to be diminished with adding paclitaxel, granting it failed to show a statistical significance.

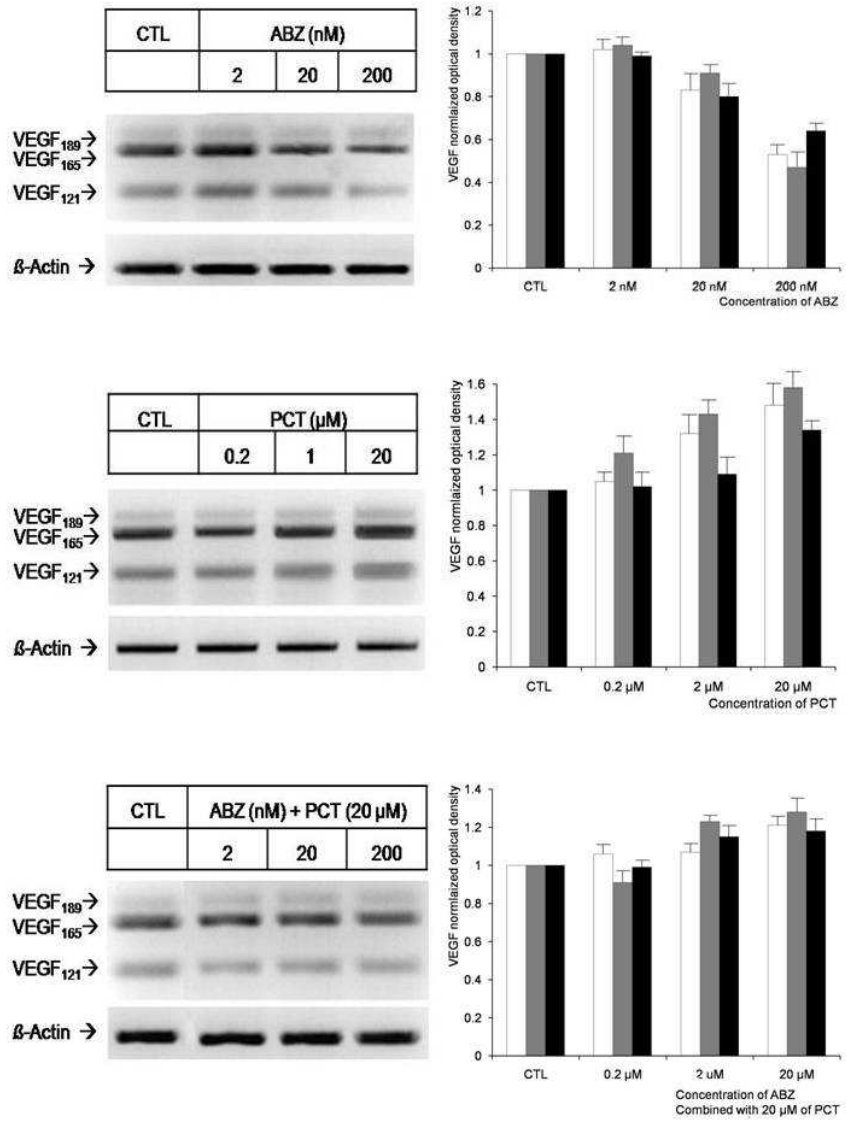

Fig. 6. RT-PCR for detection of $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$ mRNA expression in *in vitro* cultured OVCAR-3 cells with no treatment (CTL) and those treated with albendazole (ABZ), paclitaxel (PCT), and combined albendazole plus paclitaxel (COM). The ß-actin gene was used as the internal control. The products were visualized by electrophoresis on 2% agarose gel in 1× TAE buffer containing ethidium bromide. The mean

densitometry data were normalized to the results obtained from control group. The VEGF mRNAs expression from OVCAR-3 cells incubated with albendazole were diminished while those from the cells incubated with paclitaxel was increased in the dose dependent manner. Columns: mean; bars: SD.

IV. DISCUSSION

A very unique feature of ovarian cancer is its tendency to be confined to the peritoneal cavity throughout the course of disease progression. From this point of view, intraperitoneal chemotherapy was attempted and Gynecologic Oncology Group (GOG) conducted 3 large randomized phase III clinical trials of intraperitoneal chemotherapy (GOG 104, 114, and 172) that clearly showed superior progression-free and overall survival with intraperitoneal chemotherapy as compared to intravenous chemotherapy.[23]

However, most cytotoxic agents currently employed for intraperitoneal chemotherapy are well absorbed from the peritoneum leading to a rapid decline in the peritoneal drug concentrations and systemic side effects.[17] Therefore, new agents that have low absorption rates and a high hepatic clearance, which enable prolonged exposure of tumor cells to the drug, and a low systemic toxicity are needed.

Albendazole demonstrated a considerable inhibitory effect on some kinds of cancer cell lines in *in vivo* and *in vitro*.[16-21] The mechanism of the antitumor effect of albendazole was not completely elucidated; however, it had been suggested that the antitumor effect of albendazole might be related to its inhibitory effect on tubulin polymerization by competing for the colchicine-binding site and eventually blocked cell division at in the G2-M phase.[13-15] Morris et al. hypothesized that albendazole might have a tumor suppression effect and conducted a pilot study where some patients with colorectal cancer had shown a decline or stabilization of tumor marker with oral albendazole.[16] However, the antitumor effect of albendazole was much more closely related with inhibition of neovascularization rather than inhibition of cellular proliferation. More recently, Pourgholami et al. suggested that intraperitoneal administration of albendazole suppressed the expression of VEGF mRNA, inhibited angiogenesis, and exerted antitumor

and antiascitic activity.[21] Some reports indicated that a combination of VEGF inhibitor and conventional chemotherapeutic agents can significantly inhibit tumor growth and metastasis and suppress the MDR gene in tumor endothelium,[24-28] and albendazole could be a cheap and safe substitute for expensive VEGF inhibitors. Moreover, the low water and lipid solubility together with a high first-pass metabolism of albendazole—the reason for the failure of the clinical trial of oral albendazole in advanced malignancy—conversely makes albendazole attractive for intraperitoneal chemotherapy.

Consequently, we attempted to elucidate the effect of a weekly chemotherapy regimen with intraperitoneal albendazole on tumor growth and ascites formation and its possible synergistic effect with conventional cytotoxic chemotherapeutic agent in this study. Klement et al. reported that the combination of an anti-VEGFR-2 antibody with microtubule-modulating drugs shows more dramatic tumor regression than the combination of the anti-VEGFR-2 antibody with alkylating drugs[28]; Hence, we chose paclitaxel as the experimental cytotoxic agent in combination with albendazole.

We initially hypothesized that both albendazole and paclitaxel intraperitoneally administered would inhibit ascites formation and tumor growth and show a synergistic effect when administered in combination. Certainly, intraperitoneal administration of albendazole and paclitaxel significantly decreased the VEGF levels in ascitic fluid and the cumulative ascites volume in our study. However, contrary to our hypothesis, combination of albendazole and paclitaxel showed an antagonistic tendency, although the difference was not statistically significant. According to the report of Hu et al., the therapeutic effects of albendazole as an angiogenesis inhibitor and those of paclitaxel as a cytotoxic drug are considerably different.[29]

Although albendazole showed a remarkable inhibitory effect on VEGF

mRNA expression and ascites formation, it failed to suppress tumor growth. In our experiments, a macroscopic tumor mass could not be produced in 20% of the control mice; moreover, even the tumor masses that were produced in the control and treatment groups were very small. Therefore, we postulated that albendazole did not have direct tumor cell growth inhibitory effect. Albendazole, like many other VEGF inhibitors, induced the inhibition of neovascularization, thereby indirectly restricted tumor growth; cell death occurred in the cells that were most distant site from the established vasculature. Therefore, small tumors on the peritoneal surface that derive nutrients by simple diffusion or tumors already have their own vasculature are able to survive but further growth might be suppressed, and thus, they remained small.[21,22] In short, albendazole exerted its therapeutic effect mainly by inhibition of VEGF expression.

On the other hand, paclitaxel suppressed tumor growth, decreased VEGF concentration in the ascitic fluid, and inhibited ascites formation. However, because VEGF mRNA expression in the paclitaxel-treated groups was significantly up-regulated, the decrease in the cumulative ascites volume and in the VEGF levels in the ascitic fluid might be due to the direct cytotoxicity of paclitaxel to the tumor cells and tumor-related endothelium, which actively secretes VEGF, rather than the suppression of VEGF expression. Hence, we could speculate the mechanism of ascites suppression of albendazole and paclitaxel could be different : while albendazole suppressed ascites formation by inhibition of VEGF mRNA expression and paclitaxel exerted its antiascitic effects by direct cytotoxicity against VEGF-secreting tumor cells.

The most interesting finding in our report was the up-regulation of VEGF mRNA in the paclitaxel-treated groups. This finding appeared to be the cause of the antagonistic tendency in combined albendazole and paclitaxel treatment. We might be able to explain this finding on the basis of the concept of maximal toxic dose (MTD) and metronomic chemotherapy. We aimed to

mimic the clinical chemotherapy schedule as much as possible, and the experimental drugs were injected on a weekly basis. However, other reports using intraperitoneal paclitaxel generally adopted a thrice-weekly regimen. Recent studies suggested that metronomic delivery or frequent administration of some microtubule inhibitors, such as vinblastine and paclitaxel, at doses much lower than the MTD has an antiangiogenic influence and may be more effective.[30-32] Therefore, it is possible to infer that the changes in the dose and schedule of paclitaxel led to the changes in VEGF mRNA expression. Although the accurate mechanism of the antiangiogenic effect of metronomic chemotherapy is unclear, Meng et al. suggested chemotherapeutic stress with weekly 50mg/kg gemcitabine in liver cancer mouse model activated liver cancer-derived endothelial cells via nuclear factor-κB(NF-κB)-Akt-dependent manner and Akt was involved NF-κB-dependent VEGF expression.[33] More recently, Kim et al. suggested paclitaxel treatment in Hela cell could induce increased VEGF expression mediated by NF-κB activation and HIF-1α stabilization. [35] Although in many previous reports,[36-9] tumor angiogenesis could be suppressed with paclitaxel through inhibition of VEGF expression, the up-regulation of VEGF mRNA expression by weekly paclitaxel in our study might be explained in the same manner. Weekly high dose paclitaxel injection stressed directly the tumor cells or tumor-related endothelium, and this might stimulate VEGF mRNA expression via various cellular pathways. Therefore, further studies based on metronomic drug delivery schedule are warranted in order to determine whether the VEGF mRNA up-regulation by paclitaxel is directly related to the effect of MTD scheduled chemotherapy and which cellular pathways are related to the VEGF mRNA up-regulation.

In summary, weekly intraperitoneal albendazole exerted its suppressive effect on ascites formation and VEGF concentration by inhibiting VEGF mRNA expression and paclitaxel did by direct cytotoxicity on cells actively secreting VEGF, such as tumor cell or tumor related endothelium. The

paradoxically up-regulated VEGF mRNA expression by paclitaxel might be related with MTD scheduled chemotherapy stress and further study to elucidate the exact mechanism would be warranted.

V. CONCLUSION

Both albendazole and paclitaxel effectively suppressed ascites formation and decreases ascitic fluid VEGF levels in different mechanism in the OVCAR-3 cell bearing athymic nude mice model with weekly intraperitoneal administration. However, there was no demonstrable synergistic effect in combination of two drugs.

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ABSTRACT(IN KOREAN)

난소암세포를 접종한 무흉선 누드마우스에서 복강내로 투여한 Albendazole과 Paciltaxel이 복수형성과 혈관내피성장인자 발현에 미치는 효과

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최 은 경

상피성 난소암에 있어서 혈관내피성장인자는 종양의 전이와 복수 형성에 있어 중요한 역할을 담당하며 혈관내피성장인자 길항제를 난소암 환자의 치료에 사용하였을 때 통상적인 항암치료제의 효과를 높여준다는 보고들이 있다. 최근 몇몇 종양을 대상으로 한 실험에서 albendazole이 혈관내피성장인자의 발현과 복수형성을 억제하는 것으로 나타나, 이에 OVCAR-3 난소암세포주 동물모델에서 복강내 albendazole을 투약하는 것이 종양성장, 복수형성 및 혈관내피성장인자의 발현에 어떤 영향을 미치는지 알아보고 또한 대표적인 항암제인 paclitaxel과의 상승작용 여부를

알아보고자 본 연구를 계획하였다.

OVCAR-3 인간 난소암 세포를 복강내에 주입하여 암종증을 일으킨 암컷 누드마우스를 4개의 치료군으로 나누어 각기 다음의 약제를 1주에 1회 4회에 걸쳐 복강내로 주입하였다 : albendazole 단독 (450 mg/kg/wk), paclitaxel 단독 (30 mg/kg/wk), albendazole과 paclitaxel 병용(450 mg/kg/wk of albendazole 과 30 mg/kg/wk paclitaxel), 대조군(생리식염수 주입). 약물투입 일정이 종료된 후 실험동물을 치사량의 이산화탄소로 안락사시킨 후 종양의 성장, 복수의 양, 복수내의 혈관내피성장인자의 농도, 혈관내피성장인자 mRNA의 발현 정도를 각각 실험군별로 나누어 비교하였고 또한 실험실내에서 배양한 OVCAR-3 세포에 다양한 농도의 albendazole과 paclitaxel을 단독 혹은 병용하여 처리한 후 혈관내피성장인자 mRNA의 발현을 측정하여 동물실험에서의 결과와 비교하였다.

albendazole과 paclitaxel을 투여한 치료군은 모두 대조군에 비하여 복수형성과 복수 내 혈관내피성장인자 농도가 감소하는 소견을 보였으나 두 약을 병용투여하여도 상승작용은 보이지 않았다. 종양성장 억제효과는 paclitaxel 치료군에서, 혈관내피성장인자 mRNA 억제효과는 albendazole 치료군에서 더 강하게 나타났으며 paclitaxel 치료군에서는 혈관내피성장인자 mRNA 발현이 오히려 대조군에 비해 증가하는 소견을 보였다. 또한 실험실 안에서

배양한 세포주에 paclitaxel을 처리한 경우에서도 역시 paclitaxel의 농도가 증가할수록 혈관내피성장인자 mRNA 억제효과는 감소하였으며 albendazole을 처리한 경우에는 농도에 비례하여 혈관내피성장인자 mRNA의 발현은 억제되었다.

이상의 결과에서, albendazole은 혈관내피성장인자 mRNA의 발현을 억제하고 paclitaxel은 직접적으로 혈관내피성장인자를 분비하는 종양세포에 독작용을 미침으로써 복수형성을 억제하고 복수 내 혈관내피성장인자의 발현을 억제하는 것으로 생각되며, paclitaxel 치료군에서 나타난 혈관내피성장인자 mRNA의 발현증가는 배양된 난소암 세포주를 대상으로 한 연구의 결과로 미루어 볼 때 약제의 농도 및 투여방법과 관계가 있는 것으로 보여 메트로노믹 항암화학요법과 최고독성용량 항암화학요법의 항혈관생성인자효과의 차이의 기전을 이해하는 데 단서가 될 수 있을 것으로 생각된다.

핵심되는 말 : albendazole, paclitaxel, 복수,
혈관내피성장인자, 난소암