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Dienogest, a synthetic steroid, suppresses both embryonic and tumor-cell-induced angiogenesis

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Abstract

Orally administered dienogest (17α-cyanomethyl-17β-hydroxy-estra-4,9-diene-3-one) is efficacious against human hormone-dependent cancer xenografts in severely immunodeficient mice and in rats with experimental endometriosis, but its mechanisms of action remain unclear. We assessed the effect of dienogest on angiogenesis, because these two diseases that are sensitive to dienogest are known to be angiogenesis-dependent. Topical dienogest treatment dose-dependently inhibited embryonic angiogenesis, the ID_{50} value being 6.4 nmol/egg. Oral administration of dienogest (1 mg kg^{-1} day^{-1}) for 5 consecutive days significantly suppressed angiogenesis induced by S-180 mouse tumor cells in the mouse dorsal air sac assay. In vitro experiments showed that dienogest at concentrations up to 10 μM had little or no effect on the proliferation of plasminogen activator activity or formation of tube-like structures by microvascular endothelial cells. These results suggest that dienogest is a new, orally active antagonist of angiogenesis, and that its anti-angiogenic action may be involved in its therapeutic effects on cancer xenografts and endometriosis that we observed previously.

Keywords: Dienogest; Steroid, synthetic; Angiogenesis inhibition; Angiostatic therapy; Angiogenic disease

1. Introduction

Dienogest (17α-cyanomethyl-17β-hydroxy-estra-4,9-diene-3-one) is an orally active synthetic steroid which has progestational activity but not androgen-, estrogen-, anti-estrogen-, or corticoid-like activity (Katsuki et al., 1997a). Dienogest is now used for contraception (Foster and Wilde, 1998) and its application to the treatment of endometriosis is currently being studied. Our previous study showed that, in the treatment of experimental endometriosis induced by autotransplantation in rats, dienogest (0.1 mg kg^{-1} day^{-1}, p.o.) exhibits an efficacy comparable to that of danazol (100 mg kg^{-1} day^{-1}, p.o.), which is clinically as effective as medroxyprogesterone acetate (Katsuki et al., 1998). We also observed that dienogest (0.01–1 mg kg^{-1} day^{-1}, p.o.) has antitumor effects on human hormone-dependent cancer xenografts, such as endometrial and breast cancers implanted subcutaneously into severely immunodeficient mice (Katsuki et al., 1997b). The efficacy of dienogest (0.001 mg kg^{-1} day^{-1}, p.o.) against subcutaneously implanted breast cancer xenografts appeared to be comparable to that of medroxyprogesterone acetate (100 mg kg^{-1} day^{-1}, p.o.), which is used in endocrine therapy for breast cancer separately or in combination with anti-estrogens like tamoxifen.

The number of patients with breast cancers or endometriosis is steadily increasing. Thus, it is important to improve the treatment of these diseases, this being one of the major aims in this field of pharmacological research. The beneficial properties of dienogest mentioned above are noteworthy. Understanding the mechanism of action of dienogest could provide valuable information for the development of the compound as a therapeutic agent for breast cancer or endometriosis. However, little is known about its mechanism of action.

It is recognized that angiogenesis — the development of new blood vessels from preexisting vasculature — is a key event in the growth, invasion and metastasis of most cancers, including breast and endometrial cancers (Folkman, 1995; Oikawa, 1995). Furthermore, it is most likely

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that both the development and the progression of endometriosis are dependent on neovascular formation (Oosterlynck et al., 1993). Based on these findings, angiogenesis has been widely accepted to be an attractive target for improving the therapy for these angiogenesis-dependent diseases which are difficult to treat. Indeed, previous studies have shown that a number of endogenous and exogenous substances, which exhibit anti-angiogenic activity, are effective in the treatment of angiogenesis-dependent diseases such as solid tumors and rheumatoid arthritis. Interestingly, medroxyprogesterone acetate, which is a semisynthetic derivative of progesterone and has a therapeutic effect on breast and endometrial cancers, exhibits anti-angiogenic activity (Gross et al., 1981; Oikawa et al., 1988; Yamamoto et al., 1994) and also exhibits efficacy against endometriosis (Moghissi and Boyce, 1976). That dienogest is effective in animal models of these angiogenesis-dependent diseases is noteworthy. Overall, we proposed that dienogest could exert therapeutic effects on breast and endometrial cancer xenografts and endometriosis through the inhibition of angiogenesis. To verify this hypothesis, we examined the anti-angiogenic activity of dienogest in two in vivo assay systems. We also examined the effects of dienogest on the functions of vascular endothelial cells associated with angiogenesis.

2. Materials and methods

2.1. Materials

Dienogest was obtained from Jenapharm (Jena, Germany), and its chemical structure is shown in Fig. 1. Ethylene–vinyl acetate copolymer 40 was kindly donated by Mitsui-DuPont Polychemicals (Tokyo, Japan). Medroxyprogesterone acetate, MCDB 131 (Catalog Number M-8537), heparin sodium salt (low molecular weight), epidermal growth factor, bovine serum albumin, and bovine plasminogen were purchased from Sigma (St. Louis, MO). Fetal bovine serum was purchased from Moregate (Melbourne, Australia) and antibiotic (a mixture of penicillin and streptomycin) from Gibco Laboratories (Grand Island, NY). Millipore rings (inner diameter, 10 mm; thickness, 2 mm) and Millipore filters (pore size, 0.45 μm) were obtained from Nihon Millipore (Yonezawa, Japan). The culture dishes used in in vitro experiments were from Sumitomo-Bakelite (Tokyo, Japan). Matrigel was from Collaborative Biochemical Products, Becton-Dickinson Labware (Bedford, MA), and endothelial cell growth supplement was from Upstate Biotechnology (Lake Placid, NY).

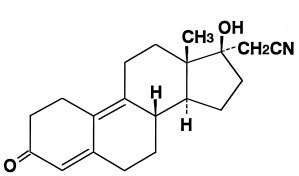

Fig. 1. Chemical structure of dienogest.

2.2. Animals

Female ICR mice aged 7–9 weeks were purchased from Charles River Japan (Atsugi, Japan). They were housed in a temperature-, air-, and light-controlled room with free access to laboratory rodent chow and water.

2.3. Cells

Malignant mouse tumor cells (S-180) were kindly provided by Dr. Chizuko Tashiro (Institute of Cancer, Tokyo, Japan) and were maintained in female ICR mice aged 8–14 weeks. Human dermal microvascular endothelial cells were purchased from Cell Systems (Kirkland, WA).

2.4. Chick embryo chorioallantoic membrane assay

The chick embryo chorioallantoic membrane assay was used for determining anti-angiogenic activity, as described previously (Oikawa et al., 1989, 1990). Briefly, the chorioallantoic membranes of 5-day-old chick embryos were treated with ethylene–vinyl acetate copolymer 40 pellets containing, or not containing, various doses of dienogest at 37°C for 2 days in a humidified egg incubator, after which an appropriate volume of a 20% fat emulsion was injected into the chorioallantois to show the vascular network better. The anti-angiogenic response was assessed as positive when the avascular zone exceeded 3 mm in diameter; only the frequency was monitored. This experiment was approved by the Committee on the Ethics of Animal Experiments of The Tokyo Metropolitan Institute of Medical Science, and was carried out in accordance with the Guidelines for Animal Experiments of The Tokyo Metropolitan Institute of Medical Science.

2.5. Mouse dorsal air sac assay

The effect of dienogest on malignant tumor-cell-induced angiogenesis was assessed by means of the mouse dorsal air sac assay, as described previously (Oikawa et al., 1997; Onozawa et al., 1997). A chamber, which was prepared by covering both sides of a Millipore ring with Millipore filters of 0.45-μm pore size, was filled with S-180 tumor cells (1×10^7 cells) suspended in 0.15 ml of phosphate-buffered saline. The S-180 tumor-cell-containing chamber was implanted into a subcutaneous dorsal air sac formed in a female ICR mouse aged 9 or 10 weeks by injecting an appropriate volume of air. The treated mice were divided into three groups; each was administered with either the vehicle (i.e., 0.5% carboxymethylcellulose sodium salt) alone ($n = 16$), 0.1 ($n = 12$) or 1 ($n = 12$) mg kg^{-1} day^{-1} of dienogest. The control group ($n = 15$),

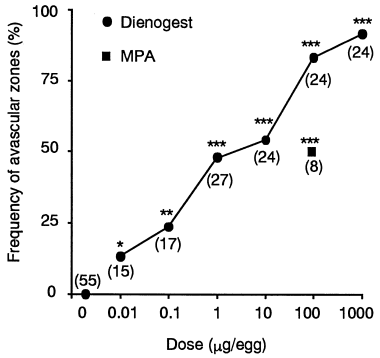

Fig. 2. Inhibitory effect of dienogest on embryonic angiogenesis. Ethylene–vinyl acetate copolymer 40 pellets containing various doses of dienogest or 100 μg/pellet of medroxyprogesterone acetate (MPA) were placed on 5-day-old chorioallantoic membranes. After 2 days, the anti-angiogenic effect was assessed by measuring the avascular zones. The points show the frequency (%) of avascular zones exhibiting a significant anti-angiogenic response. The values in parentheses are the numbers of membranes examined. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control (i.e., empty) pellet-treated membranes ($n = 55$), which did not have an avascular zone.

which received chambers containing phosphate-buffered saline in place of S-180 tumor cells, was administered the vehicle alone. Dienogest suspended in the vehicle or the vehicle alone was administered p.o. once a day for 5 days in a volume of 0.1 ml/10 g body weight from the day of implantation of the chambers. On day 5, the implanted chambers were removed from the subcutaneous fascia of the treated animals, and then a black ring with the same inner diameter as the Millipore ring was placed at the same site. The angiogenic response was assessed under a dissecting microscope by counting newly formed blood vessels of more than 3 mm in length within the area encircled by the black ring. Angiogenesis indexes 0, 1, 2, 3, 4 and 5 indicated that the number of neovessels was 0, 1, 2, 3, 4 and 5 or more, respectively. The newly formed blood vessels were morphologically distinguishable from the pre-existing background vessels by their tortuous nature, as described previously (Sidky and Borden, 1987; Plunkett and Hailey, 1990; Majewski et al., 1994; Oikawa et al., 1997). This experiment was approved by the Committee on the Ethics of Animal Experiments of The Tokyo Metropolitan Institute of Medical Science and was carried out in accordance with the Guidelines for Animal Experiments of The Tokyo Metropolitan Institute of Medical Science.

2.6. Microvascular endothelial cell proliferation

The microvascular endothelial cell proliferation assay was performed as described previously (Oikawa et al., 1998). In the presence of dienogest or medroxyprogesterone acetate, human microvascular endothelial cells (1×10^4 cells/well) were cultured in gelatin-coated 24-multiwell dishes containing 1 ml of complete medium (MCDB 131 containing 10% fetal bovine serum, 1% antibiotic, 10 μg/ml endothelial cell growth supplement, 10 ng/ml epidermal growth factor and 10 μg/ml heparin) at 37°C in a humidified 5% CO_2–95% air incubator. After 72 h in culture, the cells were trypsinized and then counted with a Coulter counter Z1 (Coulter Japan, Tokyo, Japan).

2.7. Plasminogen activator activity assay

Plasminogen activator activity derived from microvascular endothelial cells was determined as described previ-

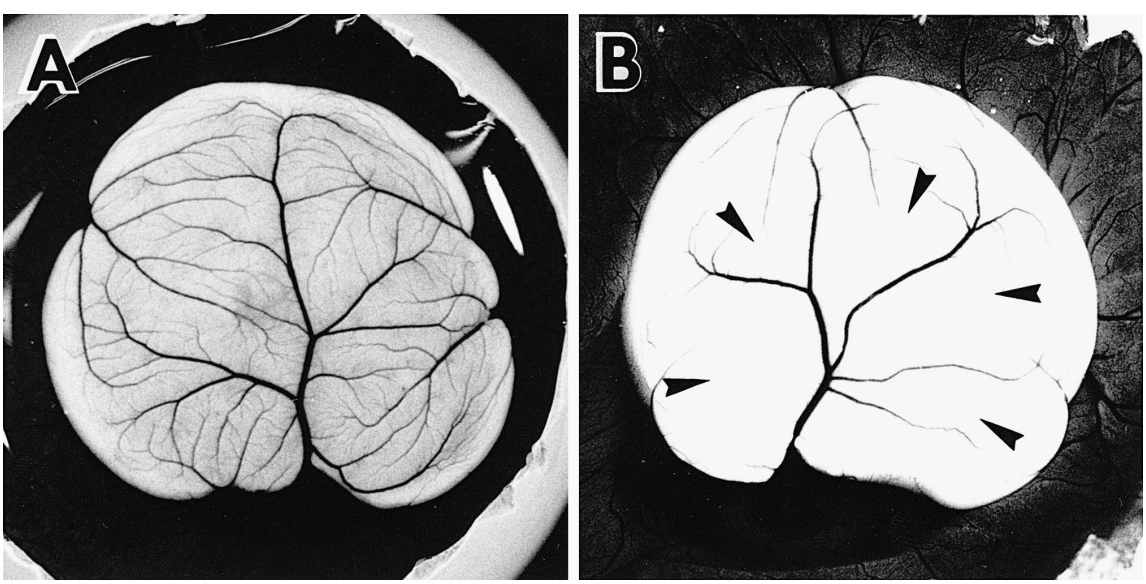

Fig. 3. Effect of dienogest on angiogenesis in chorioallantoic membranes 2 days after placement of ethylene–vinyl acetate copolymer 40 pellets impregnated with dienogest (A: 0 μg/egg; B: 1 μg/egg). An appropriate volume of a fat emulsion was injected into the chorioallantois so that the vascular network on the chorioallantoic membrane stood out against the white background of the lipid. The dienogest-containing pellet produced a significant avascular zone (indicated by arrowheads) showing anti-angiogenic activity, whereas the control pellet did not. Magnification (×2.4).

ously (Oikawa et al., 1998). In brief, microvascular endothelial cells (9×10^5 cell/dish) were cultured in gelatin-coated 35-mm dishes containing 2 ml of complete medium at 37°C for 24 h in a humidified chamber under 5% CO_2, and then incubated in MCDB 131 containing 0.1% bovine serum albumin, 10 μg/ml endothelial cell growth supplement and 10 μg/ml heparin in the presence or absence of dienogest (or medroxyprogesterone acetate) for 18 h. After collection and centrifugation of the serum-free conditioned medium, the resulting supernatant was examined for its plasminogen activator activity. Furthermore, the cells attached to dishes were washed twice with phosphate-buffered saline and then extracted with 0.5 ml of 0.5% Triton X-100 in phosphate-buffered saline. Plasminogen activator activity in the cell extracts was also determined. Plasminogen activator activity was determined at 37°C and pH 7.4 in 0.1 M Tris–HCl containing bovine plasminogen and *H*-D-Val–Leu–Lys-*p*-nitroanilide (S-2251; Chromogenix, Mölndal, Sweden). Protein concentrations were determined with bovine serum albumin as a standard according to the manufacturer's instructions (DC protein assay; Bio-Rad, Hercules, CA).

2.8. Tube formation assay

The tube formation assay was carried out as described previously (Oikawa et al., 1998). Aliquots (0.2 ml/well) of Matrigel (8.75 mg/ml) at 4°C were introduced into 24-multiwell dishes and allowed to polymerize at 37°C for about 1 h. Microvascular endothelial cells (1×10^5

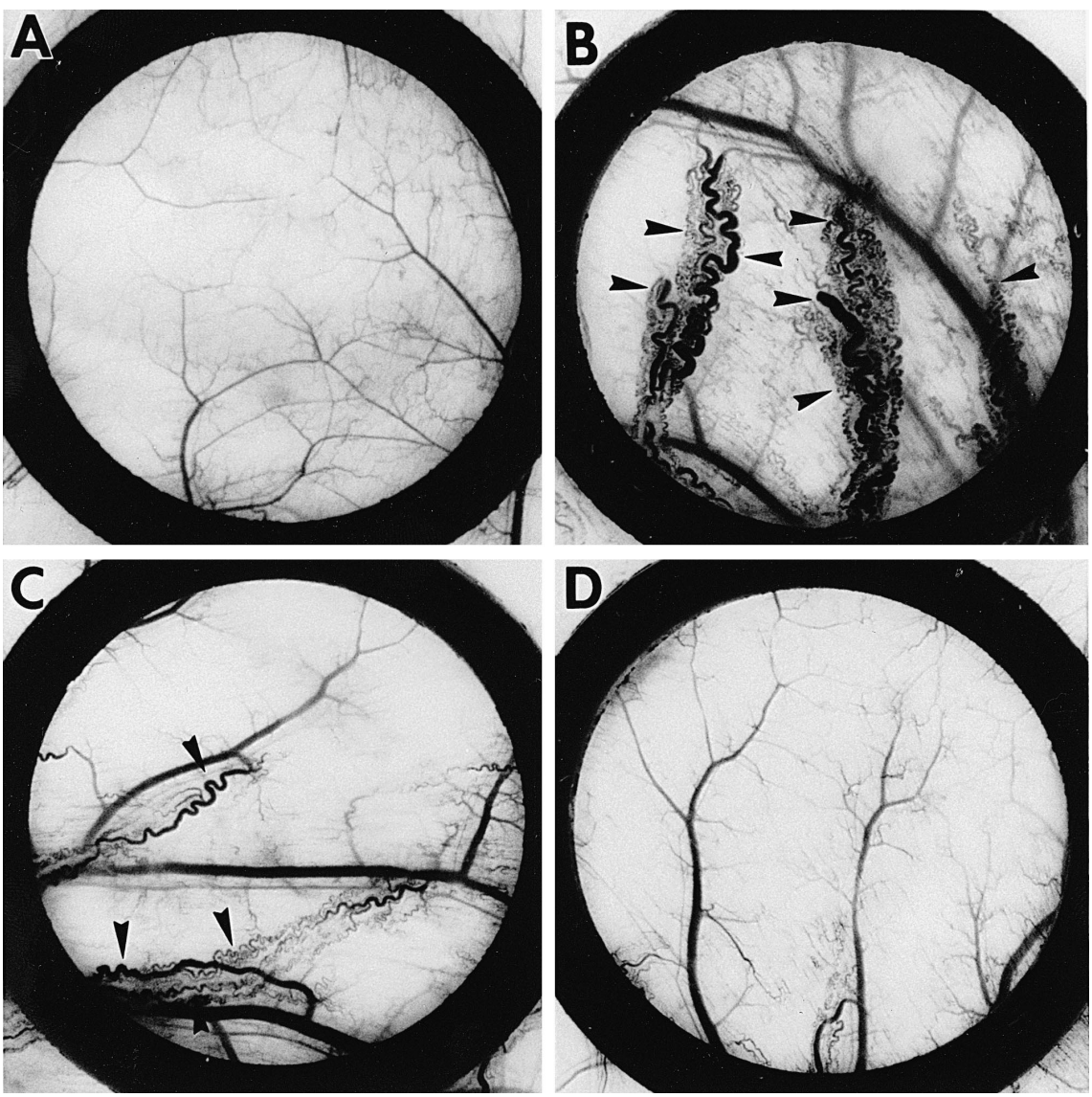

Fig. 4. Effect of dienogest on the angiogenic response 5 days after implantation of a Millipore chamber containing S-180 tumor cells. Dienogest or the vehicle was administered orally once a day for 5 days. (A) Mice implanted with a chamber containing phosphate-buffered saline were treated with the vehicle; mice implanted with a chamber containing S-180 cells (1×10^7 cells) were treated with the vehicle alone (B), 0.1 (C) and 1 (D) mg/kg dienogest, respectively. Note that dienogest (D) suppressed S-180 cell-induced formation of zigzagging neovessels (indicated by arrowheads), compared to the vehicle (B). Magnification (×6.9).

cells/well) were plated onto the Matrigel in 1 ml of MCDB 131 containing 1% fetal bovine serum and then incubated 37°C for 14 h in a humidified chamber under 5% CO_2 in the presence or absence of dienogest.

2.9. Statistics

The chorioallantoic membrane assay data were analyzed by means of Fisher's exact probability test. The mouse dorsal air sac assay data were analyzed by means of the Wilcoxon rank sum test for comparison between two groups and the nonparametric Dunnett multiple comparison procedure for comparison among three or more groups. The results of in vitro experiments are expressed as means ± S.D. and were analyzed by means of Student's *t*-test for comparison between two groups and the Dunnett multiple comparison procedure for comparison among three or more groups.

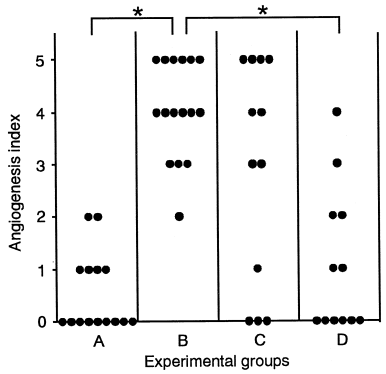

Fig. 5. Inhibitory effect of dienogest on S-180 cell-induced angiogenesis. Groups A ($n = 15$) and B ($n = 16$) received chambers containing phosphate-buffered saline and S-180 cells, respectively, and then were treated with the vehicle. Groups C ($n = 12$) and D ($n = 12$) received S-180 cell-containing chambers, followed by the oral administration of 0.1 and 1 mg kg^{-1} day^{-1} of dienogest, respectively, for 5 consecutive days. The points represent the angiogenesis index values for individual examinations. $^{*}P < 0.001$ vs. group B.

3. Results

3.1. Effect of dienogest on embryonic angiogenesis

First, we examined the effect of dienogest on embryonic angiogenesis using a system involving 5-day-old chorioallantoic membranes. Fig. 2 shows the dose–response relationship of dienogest for the appearance of an avascular zone. Topical dienogest treatment resulted in dose-dependent suppression of embryonic angiogenesis. As compared to the effect of control ethylene–vinyl acetate copolymer pellets, which did not have an anti-angiogenic effect in any of the chorioallantoic membranes tested ($n = 55$), the minimum effective dose of dienogest required for the appearance of an avascular zone was 10 ng (32 pmol) per egg ($n = 15$). The ID_{50} value for dienogest was 6.4 nmol/egg. Medroxyprogesterone acetate (260 nmol/egg), which was included as a positive control in the experiments for comparison, produced a significant avascular zone in half the chorioallantoic membranes examined ($n = 8$), which reconfirmed our previous observation (Sugino et al., 1997).

The observations in representative experiments are shown in Fig. 3. Dienogest (1 μg/egg) potently blocked embryonic neovascularization in the chorioallantoic membrane, causing a significant avascular zone (Fig. 3B), whereas the vehicle alone, as a control, had no effect in any of the chorioallantoic membranes examined ($n = 55$) (Fig. 3A).

3.2. Effect of dienogest on tumor-cell-induced angiogenesis

Next, the effect of dienogest on tumor-cell-induced neovascularization was examined in the mouse dorsal air sac assay model when administered orally. The results of representative experiments are shown in Fig. 4. The control chambers containing phosphate-buffered saline induced little or no neovascularization (Fig. 4A). The chambers containing S-180 tumor cells caused the dramatic formation of new vessels characterized by zigzag lines (Fig. 4B). The formation of these vessels was entirely suppressed by the oral administration of dienogest at a dose of 1 mg kg^{-1} day^{-1} (Fig. 4D), with weaker inhibition occurring at a dose of 0.1 mg kg^{-1} day^{-1} (Fig. 4C). These angiogenic responses were determined under a dissecting microscope by counting the tumor-cell-induced vessels with the characteristic zigzag lines (Fig. 5). The median angiogenesis index was 0.0 (range 0–2; $n = 15$) in the control group, which received phosphate-buffered saline-containing chambers, followed by treatment with the vehicle (i.e., experimental group A). The experimental group B, which received chambers containing S-180 tumor cells and then was given the vehicle, exhibited a significant angiogenic response, the median angiogenesis index being 4.0 (range 2–5; $n = 16$), compared to the experimental group A ($P < 0.001$). Compared with experimental group B, significant inhibition of the angiogenic response induced by S-180 tumor cells was observed in experimental group D treated with a higher dose of dienogest (1 mg kg^{-1} day^{-1}), the median angiogenesis index being 0.5 (range 0–4; $n = 12$) ($P < 0.001$). There was a tendency for a lower dose of dienogest (0.1 mg kg^{-1} day^{-1}) to suppress the S-180 cell-induced angiogenesis in experimental group C, although the suppression did not reach statistical significance; the median angiogenesis index was 3.5 (range 0–5; $n = 12$) ($P = 0.295$).

3.3. Effects of dienogest on the functions of vascular endothelial cells associated with angiogenesis

Angiogenesis is a consequence of a concerted sequence of events that include multiple phases, such as degradation of the extracellular matrix, and tube formation by, and

migration and proliferation of, angiogenic endothelial cells. This implies that an agent that can interfere with one or more angiogenic phases might exhibit anti-angiogenic activity. To obtain clues for understanding the mechanism of the anti-angiogenic action of dienogest, in vitro experiments involving cultured human microvascular endothelial cells were conducted to determine which function of angiogenic endothelial cells was suppressed by dienogest.

Dienogest at concentrations up to 10 μM did not have an inhibitory effect on the proliferation of or tube formation by microvascular endothelial cells (data not shown). Medroxyprogesterone acetate at a concentration of 1 μM had no effect on the proliferation of these endothelial cells.

Dienogest at concentrations up to 10 μM did not suppress the secretion of plasminogen activator activity from microvascular endothelial cells into serum-free conditioned medium, but there was no decrease in the cell-associated plasminogen activator level after the administration of dienogest (Fig. 6). In contrast, medroxyprogesterone acetate at a concentration of 1 μM, which was included in the experiments as a positive control, decreased both the extracellular and cell-associated plasminogen activator levels (Fig. 6).

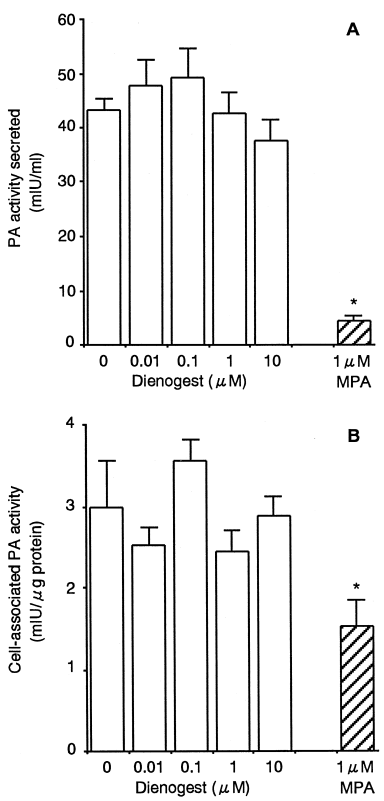

Fig. 6. Effects of dienogest on the extracellular and cell-associated plasminogen activator (PA) levels in human microvascular endothelial cells. After culture for 18 h in serum-free medium containing dienogest or medroxyprogesterone acetate (MPA), both extracellular and cell-associated PA activities were examined. The data represent means ± S.D. ($n = 4$). * $P < 0.01$ vs. control.

4. Discussion

The present study has demonstrated that dienogest exhibits anti-angiogenic activity. This novel finding verified our hypothesis that dienogest could interfere with angiogenesis. We proposed this hypothesis on the basis of our previous findings that in animal models, dienogest is efficacious against both hormone-dependent tumors and endometriosis (Katsuki et al., 1997b, 1998), which are angiogenesis-dependent diseases.

In the first experiment, the anti-angiogenic activity of dienogest was examined by means of the chorioallantoic membrane assay, because using this assay, we previously found that a variety of seemingly unrelated substances had anti-angiogenic effects. This assay clearly showed that dienogest is a new antagonist of neovascularization, the ID_{50} value being 6.4 nmol/egg. The ID_{50} value for medroxyprogesterone acetate was 260 nmol/egg, which confirmed the previous result (Sugino et al., 1997). Therefore, on a molar basis, dienogest is 40-fold stronger than medroxyprogesterone acetate in our chorioallantoic membrane assay. By contrast, dienogest seems to have weaker anti-angiogenic activity than most inhibitors of angiogenesis which we previously identified, including a synthetic retinoid Re80 ($ID_{50} = 6.3$ pmol/egg), 22-oxa-1α,25-dihydroxyvitamin D_3 ($ID_{50} = 96$ pmol/egg), herbimycin A ($ID_{50} = 260$ pmol/egg), eponemycin ($ID_{50} = 0.25$ pmol/egg), wortmannin ($ID_{50} = 70$ pmol/egg), and rhizoxin ($ID_{50} = 3.2$ pmol/egg) (Oikawa, 1995; Oikawa and Shimamura, 1996; Onozawa et al., 1997).

It has been reported that several angiostatic steroids suppress angiogenesis of chorioallantoic membranes of developing chick embryos in the presence of heparin (Crum et al., 1985). Accordingly, it is noteworthy that dienogest, an analog of steroids, had an anti-angiogenic effect in the absence of heparin. Previous studies have shown that some angiostatic steroids, including medroxyprogesterone acetate (Oikawa et al., 1988), 2-methoxyestradiol (Fotsis et al., 1994), and spironolactone (Klauber et al., 1996), do not require heparin as a cofactor for their anti-angiogenic activity.

In the second experiment, we examined the anti-angiogenic activity of dienogest when administered systemically, using the mouse dorsal air sac assay. Agents that are active in this assay have all been found to be effective in the treatment of angiogenesis-dependent diseases, including tumors and rheumatoid arthritis. The agents include TNP-470 (*O*-chloroacetylcarbamoyl-fumagillol) (Ingber et al., 1990), tecogalon (Tanaka et al., 1989), rhizoxin (Onozawa et al., 1997), and cytogenin (Oikawa et al., 1997), the first two being currently under clinical trial in patients with cancer or acquired immunodeficiency syndrome (AIDS). We used S-180 mouse tumor cells as an inducer of neovascularization because these tumor cells were shown to induce angiogenesis in the mouse dorsal air sac assay (Oikawa et al., 1997). Dienogest at a dose of 1

mg kg^{-1} day^{-1} for 5 consecutive days dramatically suppressed the angiogenic response induced by S-180 cells when administered orally. This suggests the possible involvement of the anti-angiogenic activity of dienogest in its previously observed therapeutic efficacy against human hormone-dependent cancer xenografts or endometriosis.

Because it is likely that angiostatic treatment should be given long-term, it is preferable that an anti-angiogenic agent is orally active (Kohn and Liotta, 1995; Klauber et al., 1996; Oikawa et al., 1997). Orally active inhibitors of neovascularization include carboxyamido-triazole (Kohn and Liotta, 1995), linomide (Vukanovic et al., 1993), thalidomide (D'Amato et al., 1994), irsogladine (Sato et al., 1993), 2-methoxyestradiol (Fotsis et al., 1994), tranilast (Isaji et al., 1997), and cytogenin (Oikawa et al., 1997), and some of them are currently under clinical trial in patients with angiogenic diseases like cancers (Nelson, 1998). Hence, one should note that orally administered dienogest exhibited anti-angiogenic activity in the present study and was effective in the treatment of human hormone-dependent cancer xenografts and endometriosis, diseases that are dependent on angiogenesis, as found in previous studies (Katsuki et al., 1997b, 1998).

In the third experiment, the effects of dienogest on vascular endothelial cell functions associated with in vivo angiogenesis were examined to obtain clues for understanding the mechanism of the anti-angiogenic action of dienogest. Angiogenesis is a cascade reaction consisting of multiple phases. The initial phase of angiogenesis comprises the degradation of the extracellular matrix by proteolytic enzymes like plasminogen activator and matrix metalloproteinases; thus, the inhibition of this phase is likely to lead to suppression of neovascularization. Indeed, expression and/or production of plasminogen activator have been shown to be suppressed by some antagonists of angiogenesis, including medroxyprogesterone acetate (Ashino-Fuse et al., 1989; Blei et al., 1993), irsogladine (Sato et al., 1993), genistein (Fotsis et al., 1993), and radicicol (Oikawa, 1995). With respect to chemical structure, effects on hormone-dependent tumors and endometriosis, and anti-angiogenic action, dienogest has properties similar to medroxyprogesterone acetate. Thus, we first examined the effect of dienogest on plasminogen activator production by vascular endothelial cells, and compared it with that of medroxyprogesterone acetate. Dienogest failed to suppress plasminogen activator production by endothelial cells while medroxyprogesterone acetate abolished it. This indicates that the mechanism of the anti-angiogenic action of dienogest is different from that of medroxyprogesterone acetate. Furthermore, dienogest has no effect on proliferation of or tube formation by vascular endothelial cells. Its properties are similar to those of the microbial angiogenesis inhibitor, cytogenin, we recently identified (Oikawa et al., 1997): cytogenin has no effects on the functions of vascular endothelial cells associated with in vivo angiogenesis although its oral administration suppressed S-180 tumor-cell-induced angiogenesis in our mouse dorsal air sac assay model. In this study, we failed to obtain positive data regarding the mechanism of the anti-angiogenic action of dienogest. Thus, further studies on this point are needed.

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