Retinoic acids are polyisoprenoid compounds that contain a cyclohexynyl ring and are structural and functional analogues of vitamin A. Retinoic acid can be produced in the body by two sequential oxidation steps. All-trans retinol (vitamin A) is oxidized to produce all-trans retinal (retinaldehyde), and retinaldehyde is oxidized to produce all-trans retinoic acid, which is the main biologically active form of vitamin A. Retinoids exert their effects on gene transcription by binding to intracellular receptors in the nucleus and influence cell division, differentiation, RNA, and protein synthesis [1]. Because insufficient vitamin A supply can result in immunodeficiency, anemia, and abnormal cell differentiation, researcher interest has increased in the areas of dermatology, cancer research, and embryonal development. As a result, retinoids are used in treating acute promyelocytic leukemia, and recent studies report their effects on cardiovascular disease [2,3]. In contrast, other studies report that treating endothelial cells with isotretinoin (13-cis-retinoic acid) has increased the risk of atherosclerosis and hyperlipidemia [4,5].

There is also controversy about the role of vitamin A in wound healing. Isotretinoin is conventionally used to treat acne vulgaris and psoriasis, and pretreatment with reti-
noid is widely used before dermabrasion, chemical peels, and laser resurfacing [6]. These treatments are based on the hypothesis that retinoids accelerate wound healing by promoting angiogenesis in the epidermis and dermis and affecting cells in the inflammatory phase [7,8]. In contrast, some studies report that retinoids do not affect wound healing, and others have found that they even delay wound healing [9-11].

Many recent studies about the role of retinoids in wound healing have focused on fibroblasts and keratinocytes. In this preliminary study, we focused on vascular endothelial cells, which also play an important role in wound healing and thus, the human umbilical vein endothelial cells (HUVECs) that maintain nearly all of the features of native vascular endothelial cells were used.

**Methods**

**In vitro proliferative activity of HUVECs in various concentrations of all-trans-retinoic acid (ATRA)**

We purchased HUVECs from the American Type Culture Collection (ATCC, Manassas, VA, USA) and treated with four different concentrations of ATRA (Sigma-Aldrich, St. Louis, MO, USA) from 1 to 1,000 nM (1, 10, 100, and 1,000 nM). We cultured a total of $2 \times 10^5$ cells per well in a 6-well plate in endothelial basal medium (EBM-2, Lonza, Basel, Switzerland) supplemented with 10% fetal bovine serum (FBS) at 37°C and 5% CO$_2$ for 24 hours. After 24 hours (day 0), we added 100 μL of Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) solution to each well and incubated for 1.5 hours in the incubator. We measured the absorbance at 450 nm using a microplate reader. After changing 10% FBS to 1% FBS and adding 1 mL of ATRA in four different concentrations per well, we cultured the viable cells for another 24 hours. We repeated this sequence every 24 hours in the control group and in all four experimental groups.

**In vitro proliferative HUVEC activity in RA with a wound healing promoter**

We treated HUVECs with the wound healing promoter polydeoxyribonucleotide (PDRN, Placentex Integro®, Mastelli Srl-Sanremo, Italy) in concentrations of 100 μg/mL and 1,000 μg/mL. Per well, $5 \times 10^4$ cells were cultured in EBM-2 and 0.5% FBS at 37°C and 5% CO$_2$ for 24 hours. After 24 hours (day 0), we added 100 μL of the CCK-8 solution to each well and incubated for 1.5 hours in the incubator. We measured the absorbance at 450 nm with a microplate reader. After changing 10% FBS to 1% FBS and adding 1 mL of PDRN to the viable cells in the control group and 2 mL of PDRN with 1,000 nM ATRA to the viable cells in the experimental group. Adding 100 μL of CCK-8 followed by 1.5 hours incubation and absorbance measurement and adding 2 mL of PDRN or PDRN with ATRA sequence were repeated every 24 hours.

**Statistical analysis**

We compared the average absorbance in the control and experimental groups by day after seven repeated experiments. We analyzed all data by Kruskal-Wallis test (*post-hoc* Mann-Whitney test) using SPSS version 21.0 for Windows (SPSS, Systat Software, IBM, Armonk, NY, USA). We selected a probability error of <0.05 as the criterion for statistical significance.

**Results**

**In vitro HUVEC proliferative activity in various concentrations of ATRA**

Starting from the same absorbance on day 0, the absorbance had increased until day 2 and began to decrease from day 3 in all five groups, which lasted until the end of the experiment (Fig. 1 and Table 1). Comparing the control group with each experimental group, there was statistically significant difference each day (P<0.001) except the 1 nM ATRA group. Comparing the four experimental groups, the absorbances decreased significantly as the ATRA concentrations increased (P<0.001). However, there was no statistically significant difference between 1 nM and 10 nM RA groups.

**Fig. 1.** The proliferation rates of HUVECs in various concentrations of all-trans-retinoic acid (ATRA) from 1 to 1,000 nM. (‘*’ Statistical difference with P<0.001 between every each group except between Control and ATRA 1 nM group, and ATRA 1 nM and ATRA 10 nM group.)
In vitro proliferative HUVEC activity in ATRA with the wound healing promoter

The absorbances in the four groups (100 μg/mL PDRN, 1,000 μg/mL PDRN, 100 μg/mL PDRN with 1,000 nM ATRA, and 1,000 μg/mL PDRN with 1,000 nM ATRA) had increased on day 1 (Fig. 2 and Table 2). From day 4, the two ATRA added groups began to show decreased absorbance, and the decreasing tendency lasted until the end of experiment. Compared with the 100 μg/mL and 1,000 μg/mL PDRN groups, the ATRA added groups showed significantly decreased absorbance (P<0.01). There was also a statistically significant difference between the two ATRA groups (P<0.01). However, there was no statistically significant difference between the PDRN only groups.

### Discussion

Although the effects of retinoic acid in wound healing have been discussed over 50 years, its exact effectiveness has not yet been established. Current studies have reported that retinoic acid acts on two types of nuclear receptors: retinoic acid (RARs) and retinoid X (RXRs). By binding as ligands to RARs and RXRs, retinoic acid promotes transcription of the downstream target gene into mRNA and protein, which induces epithelial proliferation and differentiation [12,13]. There is also a report that retinoic acid affects growth factors such as transforming growth factor-β (TGF-β) and insulin-like growth factor-1 (IGF-1) that induce fibroblast activation and angiogenesis [2]. However, Karadag et al. [14] reported that isotretinoin treatment had decreased the level of IGF-1 and insulin-like growth factor binding protein-3. Hung et al. [9] demonstrated that continued retinoic acid had delayed the wound healing process by prolonging the inflammatory phase and inducing capillary dilatation, intercellular space widening, and a disorganized, noncohesive epithelium. There are also studies about reti-

### Table 1. Data for absorbance of HUVECs in the presence of various concentrations of ATRA

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.793±0.008</td>
<td>1.025±0.013</td>
<td>1.310±0.016</td>
<td>1.215±0.011</td>
<td>0.967±0.009</td>
<td>0.821±0.012</td>
<td>0.728±0.011</td>
</tr>
<tr>
<td>ATRA 1 nM</td>
<td>0.793±0.008</td>
<td>1.043±0.010</td>
<td>1.214±0.011</td>
<td>1.207±0.010</td>
<td>0.983±0.005</td>
<td>0.805±0.009</td>
<td>0.725±0.011</td>
</tr>
<tr>
<td>ATRA 10 nM</td>
<td>0.793±0.008</td>
<td>1.057±0.013</td>
<td>1.198±0.011</td>
<td>1.187±0.013</td>
<td>0.963±0.010</td>
<td>0.760±0.010</td>
<td>0.702±0.010</td>
</tr>
<tr>
<td>ATRA 100 nM</td>
<td>0.793±0.008</td>
<td>1.039±0.010</td>
<td>1.130±0.010</td>
<td>1.061±0.014</td>
<td>0.809±0.009</td>
<td>0.652±0.008</td>
<td>0.606±0.008</td>
</tr>
<tr>
<td>ATRA 1,000 nM</td>
<td>0.793±0.008</td>
<td>1.007±0.008</td>
<td>1.106±0.013</td>
<td>1.011±0.012</td>
<td>0.651±0.016</td>
<td>0.512±0.007</td>
<td>0.429±0.013</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation. HUVECs, human umbilical vein endothelial cells; ATRA, all-trans-retinoic acid.

### Table 2. Data for absorbance of HUVECs in the presence of PDRN and 1,000 nM ATRA

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDRN 100 μg/mL</td>
<td>1.038±0.034</td>
<td>2.184±0.051</td>
<td>2.202±0.045</td>
<td>2.505±0.056</td>
<td>2.736±0.095</td>
<td>1.902±0.043</td>
<td>1.540±0.083</td>
</tr>
<tr>
<td>PDRN 1,000 μg/mL</td>
<td>1.038±0.034</td>
<td>2.072±0.014</td>
<td>2.154±0.024</td>
<td>2.570±0.038</td>
<td>2.703±0.058</td>
<td>2.154±0.030</td>
<td>1.656±0.018</td>
</tr>
<tr>
<td>PDRN 100 μg/mL + ATRA 1,000 nM</td>
<td>1.038±0.034</td>
<td>2.291±0.013</td>
<td>1.875±0.017</td>
<td>1.615±0.010</td>
<td>0.580±0.007</td>
<td>0.173±0.006</td>
<td>0.097±0.005</td>
</tr>
<tr>
<td>PDRN 1,000 μg/mL + ATRA 1,000 nM</td>
<td>1.038±0.034</td>
<td>2.130±0.013</td>
<td>1.764±0.010</td>
<td>1.554±0.013</td>
<td>0.611±0.006</td>
<td>0.195±0.005</td>
<td>0.094±0.003</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation. HUVECs, human umbilical vein endothelial cells; PDRN, polydeoxyribonucleotide; ATRA, all-trans-retinoic acid.
Effect of vitamin A on endothelial cells

Retinoic acid (RA) is an endogenous ligand of the retinoic acid receptor (RAR) and retinoid X receptor (RXR) that regulates cell proliferation, differentiation, and apoptosis. RA has been shown to affect the wound healing process, and a plethora of studies have indicated that RA may delay wound healing, especially in large wounds and chronic wounds. This study aimed to analyze the effects of RA on vascular endothelial cells. We investigated RA's effect on the proliferation of vascular endothelial cells (HUVECs) in vitro.

We analyzed the effects of retinoic acid (RA) in various concentrations from 0 to 1,000 nM on HUVECs. We evaluated the proliferative activity of HUVECs using a proliferation assay. RA had concentration-dependent suppression of fibroblast activation, with RA concentrations of 10 nM to 1,000 nM significantly suppressing HUVEC proliferation activity compared to the control group. However, there were no significant differences in the proliferation of HUVECs exposed to RA concentrations of 0 to 10 nM.

In conclusion, ATRA in concentrations from 10 nM to 1,000 nM has definitely suppressed the proliferation of vascular endothelial cells in vitro. Because vascular endothelial cells play a key role in coagulation, hemostasis, and angiogenesis in wound healing, retinoic acid may delay wound healing in such conditions.

Conflict of interest

No potential conflicts of interest relevant to this article are reported.
References