# Anti-atherosclerotic effect of *3*-hydroxybenzaldehyde

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# Anti-atherosclerotic effect of *3*-hydroxybenzaldehyde

**Directed by Professor Eun Jig Lee**

**The Master's Thesis**

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**Master of Medical science**

**Soojung Im**

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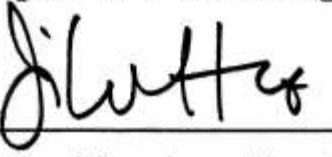
**This certifies that the Master's Thesis**

**of Soojung Im is approved.**

**Thesis Supervisor : Eun Jig Lee**



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**The Graduate School**

**Yonsei University**

**December 2014**

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이 밖에도 제가 미처 언급하지 못한 모든 분들께 감사드립니다.

제가 있을 수 있게 길을 준비해 두신 주님, 감사드립니다.

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## ABSTRACT

### Anti-atherosclerotic effect of *3*-hydroxybenzaldehyde

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(Directed by Professor Eun Jig Lee)

Atherosclerosis is a complex vascular disease caused by thickening arterial wall, infilteration of leukocytes, abnormal proliferation and migration of vascular smooth muscle cells (VSMCs) and the formation of multiple atheromatous plaques within arteries.

*3*-Hydroxybenzaldehyde (*3*-HBA) is one of the three isomers of hydroxybenzaldehyde and water-soluble phenolic aldehyde synthesized chemically, but its therapeutic function is unknown. In this study, the inhibitory role of *3*-HBA on platelet derivative growth factor-induced migration and proliferation in VSMCs was studied. In addition, *3*-HBA diminished signal-transduction involved in the inhibition of platelet derivative growth factor-induced migration and proliferation. Moreover, *3*-HBA attenuated platelet aggregation in both *in-vivo* and *ex-vivo* conditions and prevented tail vein thrombosis induced by κ-carrageenan injection. In these experiments, *3*-HBA reduced blood aggregating velocity and length of thrombus in the tail of rats. These findings suggest a potential therapeutic role of *3*-HBA in the treatment of atherosclerosis.



Key word: atherosclerosis, *3*-Hydroxybenzaldehyde, VSMC, PDGF, migration, proliferation, antithrombotic

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## I. INTRODUCTION

Atherosclerosis is a complex disease that arterial walls become thickened and hardened by increased intima size. The accumulation of leukocytes and residues form atheroma and intensify the pathological disorder. Also repetitive vascular smooth muscle cells (VSMCs) migration and proliferation aggravate sclerosis.[1-3]

Atherogenesis and intima hyperplasia cause various cardiovascular disorders, including heart failure, myocardial infarction and cerebral infarction.[1,2,4] When the inner layers of arteries are injured, various cell types including endothelial cells, secrete cytokines and growth factors. These multiple factors such as platelet-derived growth factor (PDGF) induce VSMCs to change from quiescent state to synthetic state.[1,5] PDGF is significant regulating factor of cell cycle of VSMCs, and also encourage the

movement.[2] Thus PDGF stimulation increases the factors involved in proliferation of VSMCs and it activates matrix metralloproteinases (MMPs) to break down extracellular matrix to move.[3,6] It is also known as an activator of protein kinase B (AKT) through the activation of Ras and phosphatidylinositol *3*-Kinase (PI3K)[7-9] As well as intima hyperplasia, atherothrombosis is fibrous wastes from inner arterial layer coagulate together and form platelet thrombi lead to vascular occlusion.[2,6,10] Patients are prescribed an anticoagulants or antiplatelet drugs because of high relapsing possibility of this disease.[11]

Protocatechuic aldehyde (PCA; *3,4-di*hydroxybenzaldehyde) is a chemical compound that is found from various natural components such as Danshen which has been known as natural medicine for atherosclerosis.[2,12] PCA is known as an inhibitor of proliferation and migration by PDGF in VSMCs and an anticoagulant. Unlike the limelight received PCA, *3*-hydroxybenzaldehyde is not reported for its therapeutic effectiveness and it also has structure that is lacking one hydroxyl group from PCA. In this study, we discovered that *3*-hydroxybenzaldehyde inhibits proliferation and migration of VSMCs causing intimal hyperplasia as well as platelet aggregation in *in-vivo* condition.

## II. MATERIALS AND METHODS

### 1. Production of *3*-hydroxybenzaldehyde

*3*-hydroxybenzaldehyde (*3*-HBA) was purchased from SIGMA-Aldrich (St. Louis, MO, USA). The powder was dissolved in 60 ˚C.-warmed distilled water for 2 hours with a rotary evaporator. Next, the solution was filtered by 0.2 μm pore cellulose acetate syringe filter (Satorious, Goettingen, Germany) diluted to final experimental concentrations. The solution was warmed to 60 ˚C every time to use.

### 2. Primary cell isolation and culture

Rat vascular smooth muscle cells (VSMCs) were isolated from the thoracic aorta of 6 weeks SD male rats (Orient-Charles River Technology, Gyeonggido, Korea) and stored in liquid nitrogen after suspended in freezing medium (feral bovine serum with 10% DMSO). All experiment, VSMCs were utilized between passage 4 to 6. Cells were grown in Dulbecco's High Glucose Modified Eagles Medium (Hyclone, Logan, UT, USA) with 10% of fetal bovine serum. For synchronization, cells were starved by same media without serum for 24 hours. After synchronization, cells were treated with *3*-HBA for 24 hours prior to stimulation with platelet-derived growth factor (PDGF)-BB (R&D Systems, Minneapolls, MN, USA) for 24 or 48 hours up to the experiments.[2,12,15]

### 3. Cell viability and proliferation assay

After seeded 5 x $10^3$ cells per well of 96 cell culture plate, cells are stabilization in 200μl DMEM with 10% serum for a day and synchronize in 100μl DMEM without serum for a day. As treatment of *3*-HBA (0, 25, 50, 100 μM), cells are exposed to media with

PDGF (25 ng/ml) for 24, 48 or 144 hours depending on the groups. The cells were subjected to the 10μl of Cell Titer 96® AQ ueous Non-Radioactive MTS Cell Proliferation Assay (Promega, Madison, WI, USA) for 4 hours and the absorbance is read at a wavelength of 490 nm. The percentage of cell growth was evaluated by setting as 100% the growth of control cells treated only media.[2]

Proliferation was also determined by bromodeoxyuridine (BrdU) incorporation assay (Cell Proliferation ELISA; Roche, Basel, Switzerland). Cells were seeded as same as MTS assay, BrdU incorporation analysis was performed according to the recommended instructions. The absorbance was measured at 450 nm.[2,12]

4. Propidium iodide staining for cell cycle analysis

Propidium Iodide was purchased from SIGMA-Aldrich and prepared 500 $\mu$g/ml in PBS containing 10% FBS. Cells were collected by tripsinazation and resuspended in 0.3 ml PBS containing 10% FBS. Cells were fixed with pre-cold pure ethanol for over 2 hours at – 20 ˚C, after twice of wash, stained in PI staining solution at room temperature for 30 minutes. Cell cycle analysis was evaluated with a flow cytometer (FACS Calibur, BD Biosciences, San Jose, CA, USA) and analyzed by flow-jo program.[2]

5. Reverse transcription and quantitative real-time PCR (RT-PCR)

Total RNA was extracted from cell lysates with Isol RNA lysis reagent (5 prime, Hilden, Germany) and cDNA was prepared using ReverTra Ace -α- ®(Toyobo, Osaka, Japan). All steps were was performed according to the recommended instructions. Oligonucleotide primers were synthesized from Bioneer (Seoul, Korea) and steps were followed by 30 cycles consisting denaturizing at 95˚C for 30 sec, annealing at 56˚C for

30 sec, and polymerizing at 72 ˚C for 5 min, followed by additional polymerizing at 72˚C for 5 min. Glyceraldehyde *3*-phosphate dehydrogenase (GAPDH) was used as a loading control. PCR products were observed in 2% agarose gel electrophoresis. Amplified products were electrophoresed on 2% agarose gel with SafeView from NBS biologicals (Cambridgeshire, UK) and observed by ultraviolet trasnilluminator. [8,9]

6. Immunoblotting

Cell lysates were prepared with RIPA buffer (Biosesang, Kyeonggido, Korea) containing phenymethylsulfonyl fluoride (1 mM), sodium fluoride (50 mM), sodium orthovanadate (0.2 mM), β-glycerophosphate (1 Mm) and protease inhibitor cocktail. Each chemical was purchased from Sigma-Aldrich except protease inhibitor cocktail (04693159001, Roche, Basel, Switzerland). Membranes were then immunoblotted with primary antibodies for phosphor-Akt (Cell Signaling, Beverly, MA, USA), Akt (Cell Signaling) and MMP-2 (Santa Cruz biotechnology, Santa Cruz, CA, USA). Every primary antibody was diluted 1:1000 in tris-buffered saline containing 0.05% tween 20 and with 5% bovine serum albumin. [8,9] Peroxidase-conjugated anti-rabbit or anti-mouse antibodies were used as secondary antibodies (Thermo Fisher Scientific, Rockford, IL, USA). β-actin was used as a loading control.[2,12]

7. Cell migration assay

Upon VSMC reaching 80% of confluence in 6 well plate, the cell layer was scratched with a sterile plastic 1000 μl tip. Cell migration was photographed using a Nikon microscope system (Nikon Instruments, Inc., Melville, NY, USA). The area of wound healing was measured using Image J program (NIH).

8. Blood aggregation

This study was performed with the approval of Institutional Animal Care and Use Committee of Yonsei University Health System based on the Laboratory Animal Manual and the "Guide for the care and use of laboratory animals" edited by the National Research Council of the National Academies (permit number 2012-0022-2). All animal studies were performed in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care. All animal models were anesthetized with an intraperitoneal injection of Rumpun-Zoletile mixture once every procedure.

The sample blood for *ex-vivo* was taken from two 6-week male Sprague Dawley rats (Orient-Charles River Technology, Gyunggido, Korea) and collected with heparin (1.0%, JW Pharmaceutical, Seoul, Korea) to prevent precoagulation and stored on ice. Also sample blood for *in-vivo* test was taken after the animals were pretreated *3*-HBA and aspirin (100 mg/kg/d, n=6 in each groups) for 1 weeks via i.p. injection. The group of control was injected normal saline (JW Pharmaceutical, Seoul, Korea). Platelet aggregation was evaluated with an impedance aggregometer (Chrono-log model 700, Chronsolog Corporation, Havertown, PA, USA) and induced by ADP (20 μM, Sigma–Aldrich Co.)[2,12]

9. SD rat models for tail vein thrombosis

Tail thrombosis was induced in 6-week male SD rats to evaluate the effect of *3*-HBA on thrombosis formation. Rats were grouped into three (n=6), and in a group were injected *3*-HBA (100 mg/kg) once a day for a week intraperitoneally. The other groups were injected with the same volume of normal saline (JW Pharmaceutical, Seoul, Korea) as a

vehicle. All experimental animals were injected κ-carrageenan (1 mg/kg) via tail vein injection intravenously. After injection, the locations of 13 cm from tail tips were ligated for 10 minutes and untied. In a group which injected with vehicle were injected heparin (200 IU, JW Pharmaceutical, Seoul, Korea) intraperitoneally after 10 minutes from κ-carrageenan injection.[12,16]

## III. RESULTS

### 1. VSMC mortality after *3*-hydroxybenzaldehyde treatment

The cytotoxicity of *3*-HBA (Figure 1) was measured through cell viability using MTS assay in a dose-dependent manner. More than 90% of cells were viable after treatment with 12.5 – 400 μM of *3*-HBA and the survival rate was over 50% at all concentration. (Figure 2)

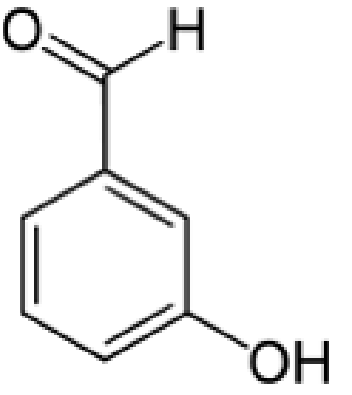



**Figure 1.** Chemical structure of *3*-HBA

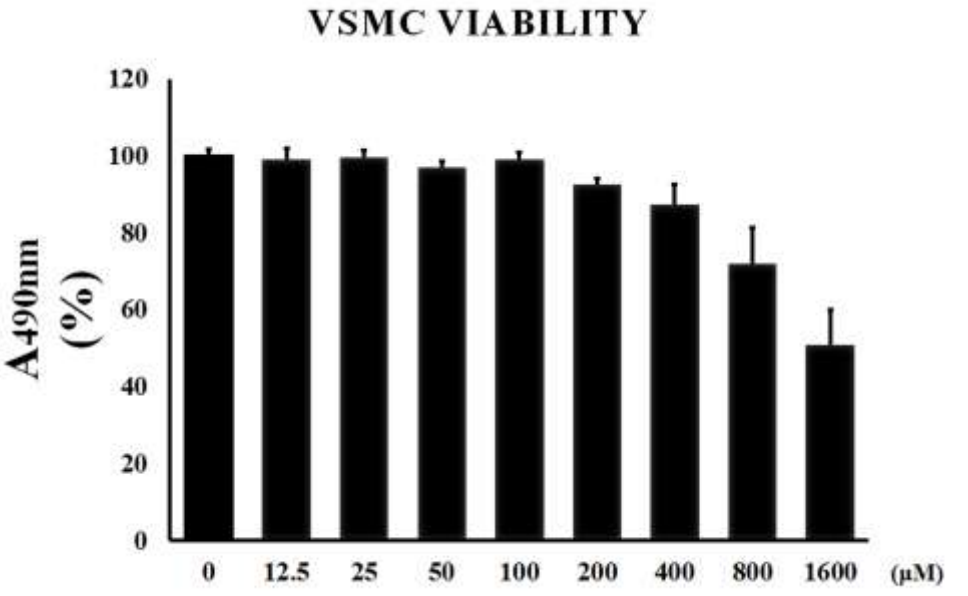



**Figure 2.** The effect of *3*-HBA on viability of rat VSMCs. VSMCs were starved for 24 h and treated with indicated concentration of *3*-HBA for 48 hours. To investigate the effect of *3*-HBA on the cell of rat VSMCs, an MTS assay was performed. Each value represents the absorbance at 490nm in cells compared to that in vehicle-treated cells which is arvitrarily denoted as 100%.

**2.** The inhibitory effect of *3*-HBA on proliferation of rat VSMCs

MTS assays were performed to confirm that *3*-HBA suppresses VSMC proliferation induced by PDGF stimulation. Cell proliferation was inhibited by *3*-HBA in a dose-dependent manner for up to 144 hours of treatment (Figure 3**A**). BrdU incorporation assay was used to determine the rate of DNA synthesis. BrdU incorporation was greater on PDGF treated cells, while the result was reduced significantly on *3*-HBA pretreated cells (Figure 3**B**). Also PDGF induces phosphorylation of AKT that is known as a modulator of various gene regulator protein activation was also inhibited *3*-HBA treatment (Figure 3**C**).

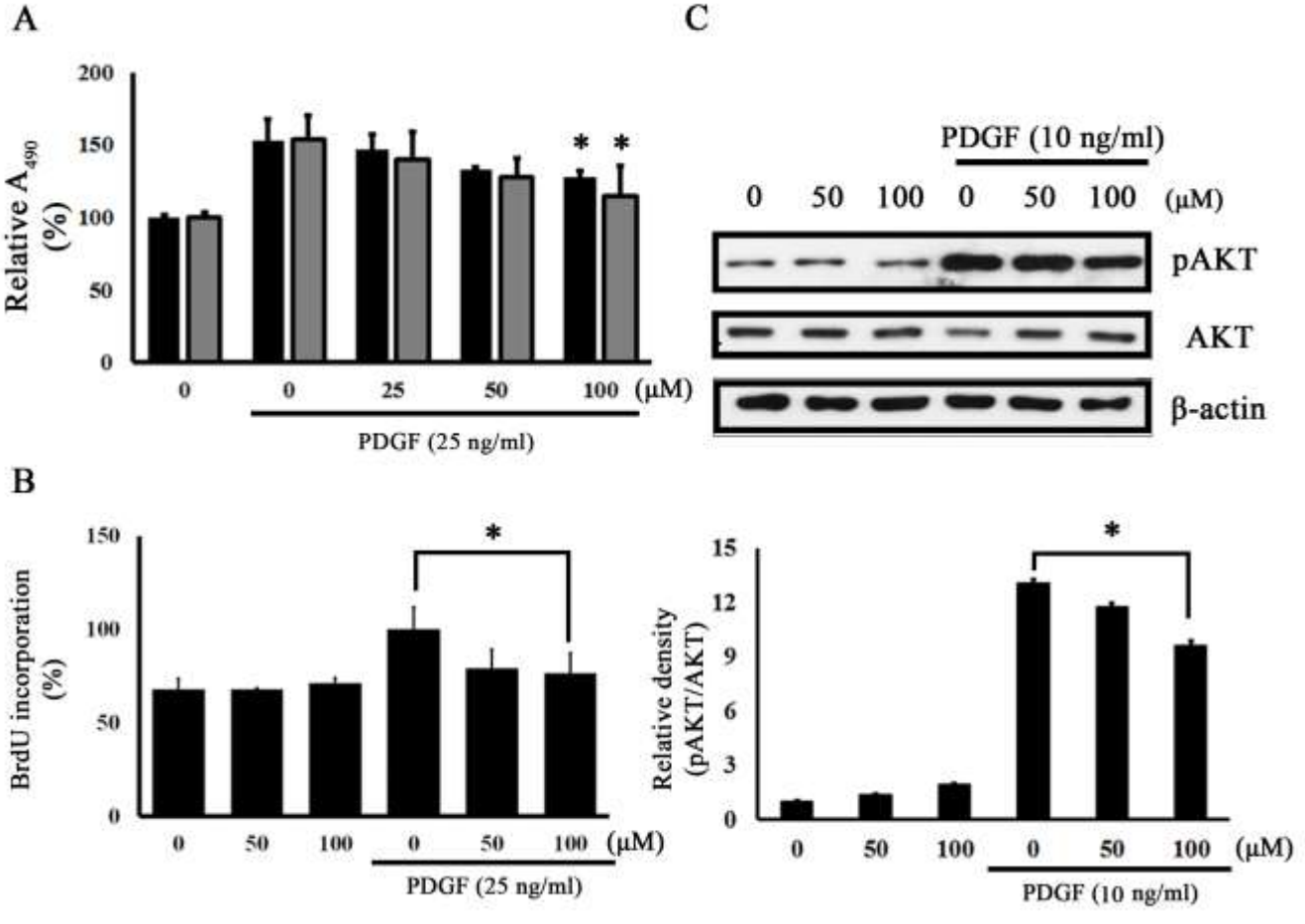

A
Relative A490 (%)
200
150
100
50
0
0
0
25
50
100
(μM)
PDGF (25 ng/ml)
*
*
C
PDGF (10 ng/ml)
0
50
100
0
50
100
(μM)
pAKT
AKT
β-actin
B
BrdU incorporation (%)
150
100
50
0
0
50
100
0
50
100
(μM)
PDGF (25 ng/ml)
*
Relative density (pAKT/AKT)
15
12
9
6
3
0
0
50
100
0
50
100
(μM)
PDGF (10 ng/ml)
*

**Figure 3.** The inhibitory effect of *3*-HBA on PDGF-induced proliferation of rat VSMCs. (**A, B, C**) VSMCs were starved serum for 24 hours and were pretreated 0, 25, 50, 100μM *3*-HBA for 24 hours. (**A**) Cells were stimulated with 10 ng/ml PDGF for 48 and 144 hours. Media was changed every 48 hours. Cell proliferation was measured with MTS-based assay, observed at 490 nm. (**B**) Cells were stimulated with 25 ng/ml PDGF for 48 hours and labeled by BrdU. (**C**) Cells were stimulated with 10 ng/ml PDGF for 24 hours. Western blots are demonstrating the effects of PDGF and *3*-HBA phosphorylated AKT (pAKT) from whole cell lysate. β-actin used as a loading control. Each results are expressed as the mean ± SEM (standard error of the mean). * represents statistical significant differences as compare to the group treated with PDGF alone ($p < 0.05$).

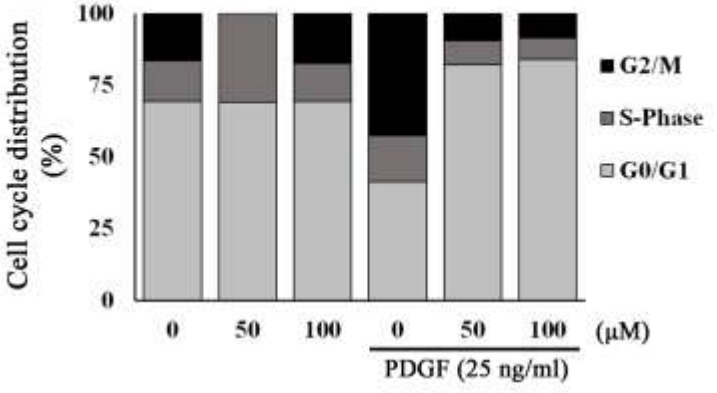
**3.** The inhibitory effect of *3*-HBA on cell cycle of rat VSMCs.

In VSMCs cell cycle, *3*-HBA treatment decreased the number of cells at S and G2/M phase by PI staining (Figure 4**A).** In particularly, the results were observed that mRNA expression levels of CD1 and Rb1, which regulate cell cycle, were inhibited after *3*-HBA treatment compared to in group treated with PDGF alone (Figure 4**B**). mRNA expression was measured by reverse transcription and quantitative real-time PCR (RT-PCR). Primers for *ccnd1* and *rb1* gene describe in Table 1. To conclude, *3*-HBA arrests S and G2/M phase of cell cycle through Rb1 and CD1 on VSMCs.

**Table 1.** the sequences of primers

| Gene name | | Sequence | Product size |
|---|---|---|---|
| *Ccnd1* | Forward | CCTGACTGCCGAGAAGTTGT | 165 |
| | Reverse | TCATCCGCCTCTGGCATTTT | |
| *Rb1* | forward | AACTCTGGGGCATCTGCATC | 441 |
| | reverse | TTGCAGCTGTTTTGTACGG | |

A


Cell cycle distribution (%)
100
75
50
25
0
G2/M
S-Phase
G0/G1
0
50
100
0
50
100
(μM)
PDGF (25 ng/ml)

B

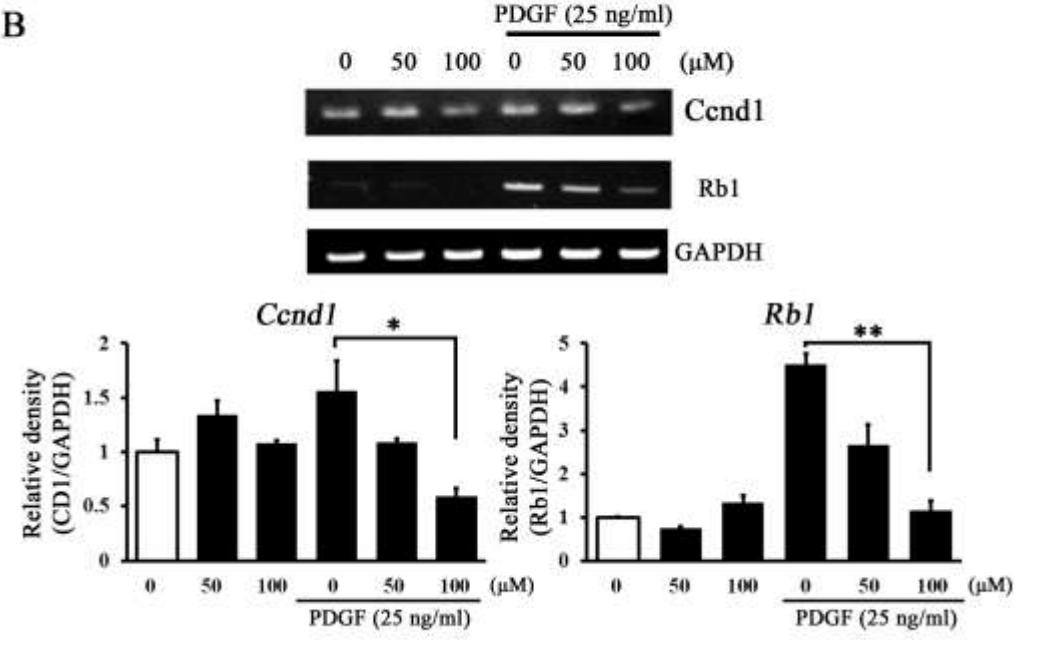
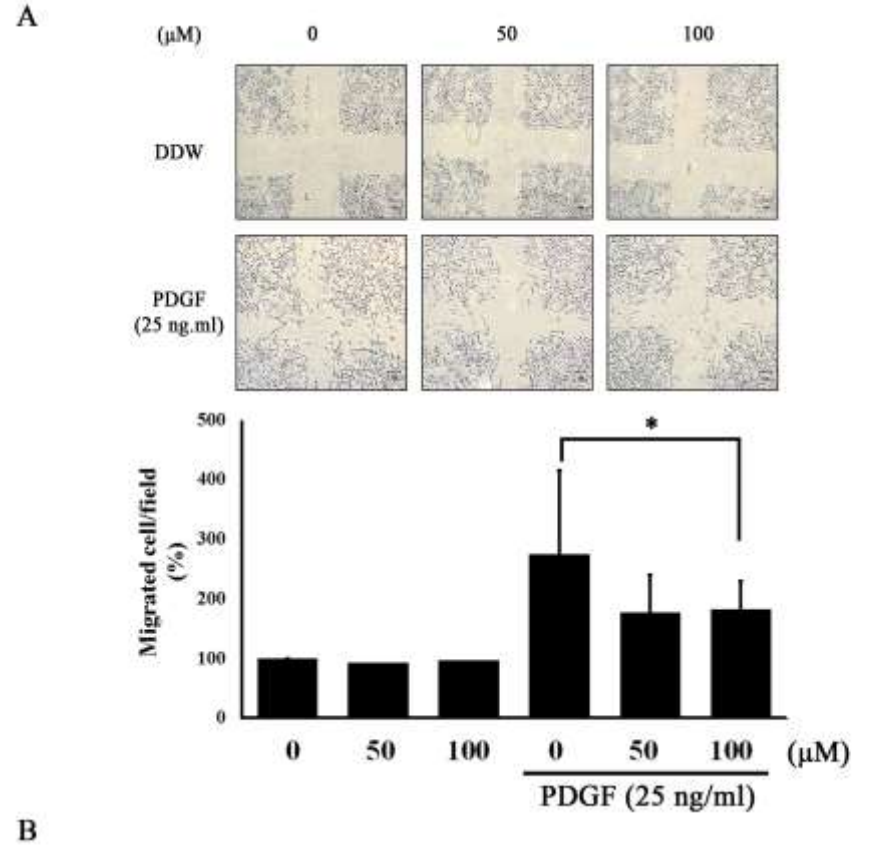

PDGF (25 ng/ml)
0
50
100
0
50
100
(μM)
Ccnd1
Rb1
GAPDH
Ccnd1
*
Relative density (CD1/GAPDH)
2
1.5
1
0.5
0
0
50
100
0
50
100
(μM)
PDGF (25 ng/ml)
Rb1
**
Relative density (Rb1/GAPDH)
5
4
3
2
1
0
0
50
100
0
50
100
(μM)
PDGF (25 ng/ml)

**Figure 4**. The inhibitory effect of *3*-HBA on cell cycle of rat VSMCs. (**A, B**) VSMCs were starved in medium lacking serum for 24 hours, and then pretreated at indicated concentration of *3*-HBA for 24 hours. VSMCs were then stimulated with PDGF (25 ng/ml) for 24 hours. (**A**) Cell cycle distribution was measured with PI staining. *3*-HBA treatment decreased the number of cells at S and G2/M phase. (**B**) Gene expressed level was analyzed by reverse transcription and quantitative RT-PCR. mRNA expression levels of CD1 and Rb1 gene were inhibited after *3*-HBA treatment. Each result is expressed as the mean ± SEM. * and ** indicate $p<0.05$ and $p<0.005$ compared to control group, respectively.

**4.** The inhibitory effect of *3*-HBA on PDGF-induced migration of rat VSMCs**.**

As assessment on migration of VSMCs is important criteria to evaluate the effect of *3*-HBA for treatment of atherosclerosis we have performed wound-healing experiments. In a dose dependent manner of *3*-HBA inhibited the migration of VSMCs (Figure 5**A**). In accordance with the image, the graph showed that *3*-HBA decreased the number of migrated cells. Moreover, the expression of MMP-2, an important protein marker for the migration of cell, also decreased significantly in *3*-HBA treated group (Figure 5**B**).


A
(μM)
0
50
100
DDW
PDGF
(25 ng.ml)
Migrated cell/field
(%)
500
400
300
200
100
0
*
0
50
100
0
50
100
(μM)
PDGF (25 ng/ml)

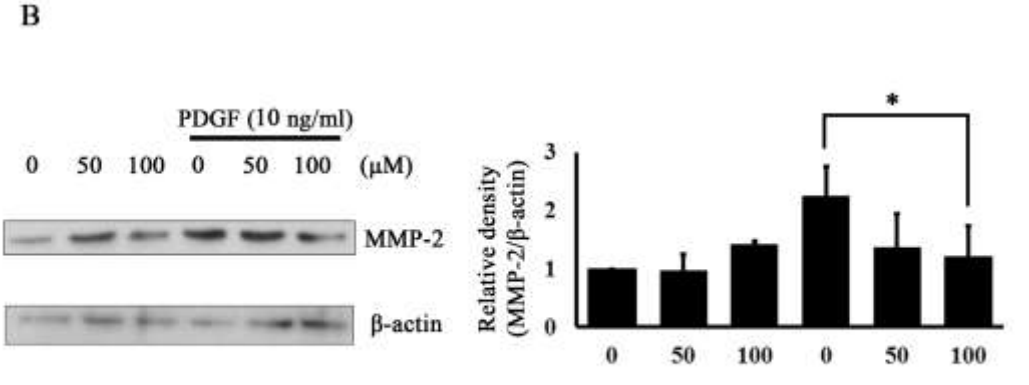

B
PDGF (10 ng/ml)
0
50
100
0
50
100
(μM)
MMP-2
β-actin
Relative density
(MMP-2/β-actin)
3
2
1
0
*
0
50
100
0
50
100

**Figure 5.** The effect of *3*-HBA on migration of rat VSMCs. (**A, B**) VSMCs were serum starved for 24 hours, and then pretreated with *3*-HBA (0, 50, 100 μM) for 24 hours. VSMCs were stimulated with PDGF (**A**) 25 ng/ml and (**B**) 10ng/ml for 24 hours. (**A**) Before stimulation, wells were scratched, cells were stained by hematoxylin and scored by Image J program. VSMCs were inhibited from migrating in a dose dependent manner of *3*-HBA. (**B**) Protein expression of MMP-2 was determined by western blot analysis. The expression of MMP-2 decreased significantly in *3*-HBA treated group. β -actin was used as a loading control. Each result is expressed as the mean ± SEM. * indicates $P<0.05$ compared to the PDGF treated group.

**5.** The anti-ADP-induced blood aggregation effect of *3*-HBA on SD rats

To evaluate inhibitory effect of *3*-HBA on the rat platelet aggregation, *ex-vivo* and *in-vivo* experiments were performed. For the *ex-vivo* experiment, the sample blood was collected from SD rat whole blood and mixed with *3*-HBA before aggregation assay. *3*-HBA decreased the aggregation velocity dose-dependently in ADP (20 μM)-induced platelet aggregation (Figure 6**A**). For the *in-vivo* experiment, *3*-HBA was introduced to rats for 1 week. *In-vivo* result shows that *3*-HBA has potential capability as an anti-platelet like aspirin (Figure 6**B**).

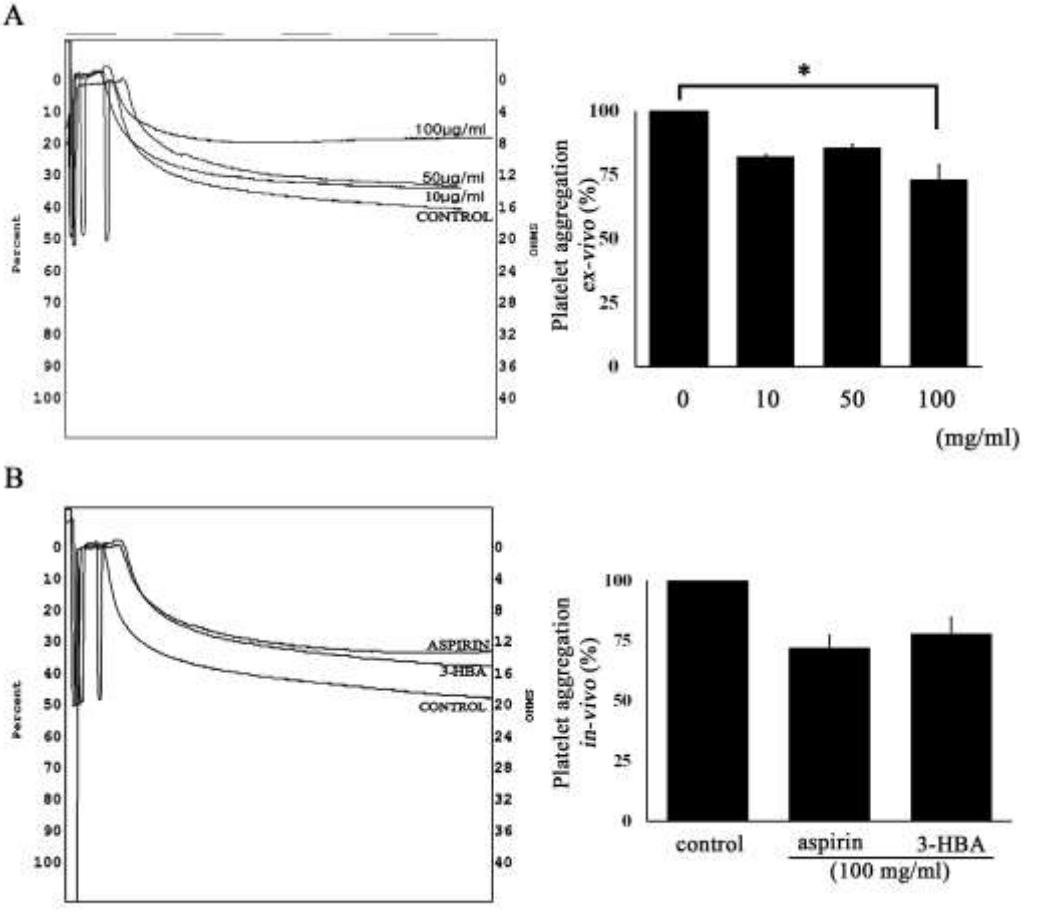



**Figure 6.** The blood thinning and restoration effect of *3*-HBA on SD rat. (**A, B**) The sample blood was taken from 7-week-male SD rats. Platelet aggregation after *3*-HBA treatment (**A**) *ex-vivo* condition and (**B**) *in-vivo* condition. (**A, B**) The platelet aggregation was induced by ADP. *3*-HBA decreased the aggregation velocity dose-dependently in platelet aggregation. (**A**) *3*-HBA and aspirin administered at a dose of 100 mg/kg/ for 1 week by i. p. injection ($n$=6 in each group). the aggregation velocity decreased *3*-HBA treated as much as aspirin in platelet aggregation. (**B**) Results are expressed as the mean ± SEM. * indicates statistical differences versus control group, respectively ($p<0.05$).

**6.** The restoration effect of *3*-HBA on κ-Carrageenan-induced tail vein thrombosis SD rat models

To evaluate anti-thrombosis effect of *3*-HBA, Bekemeier's modified tail vein thrombosis model was performed. Three different SD rat groups were injected with substances for one week and utilized for this experiment, which are *3*-HBA (100 mg/kg, n=6), positive control (heparin 200 IU, n=6), and sham-operated (normal saline, n=6). For positive group, normal saline was given like sham-operated groups, but heparin was injected to rats once along with 1mg/kg κ-carrageenan injection. Representative picture shows average thrombotic region of each group after 72 hours of Bekemeier's model performed. Black line indicates the position of tail 13 cm from tail tip. At this position, tail was tied when tail vein thrombosis model was induced .The graph represents the length of thrombotic region throughout the different time interval (Figure 7). The length of thrombotic region were decreased the *3*-HBA treated group as much as the heparin injected group.

control heparin (200 IU) 3-HBA (100 mg/ml)

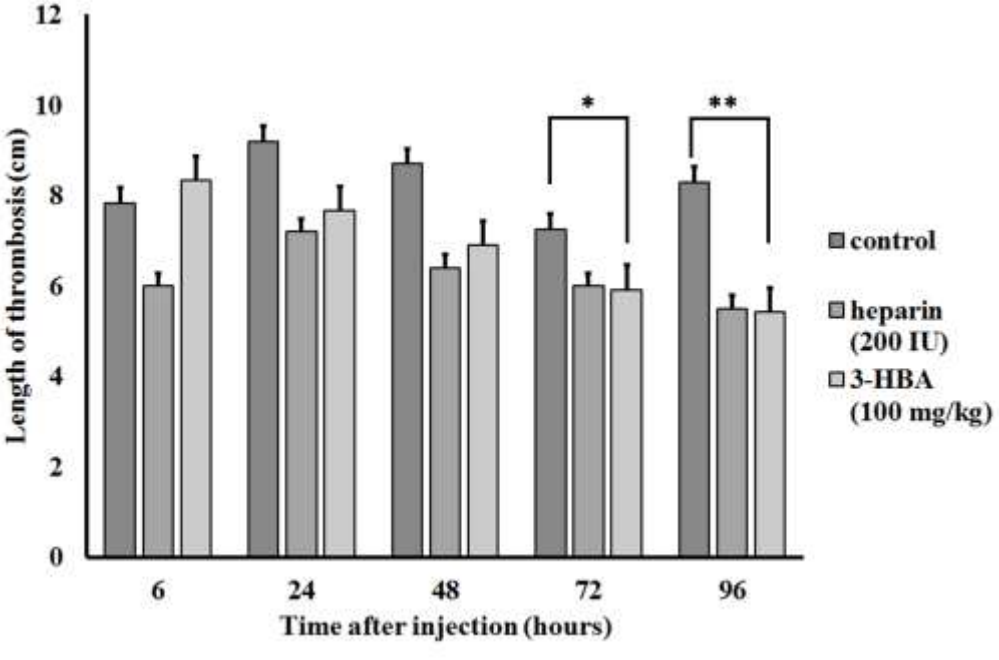


**Figure 7.** The effect of *3*-HBA on tail vein thrombosis SD rat models**.** *3*-HBA (100 mg/kg/d) administered for 1 week via i. p. injection and heparin (200 IU) was administered once via i.p. injection. The pictures represent gross changes in the tail vein 72 hours after κ-carrageenan injection. The black bar on the picture indicates the position 13 cm from the tail tip. The dot graph represents change of gross length. Results are expressed as the mean ± SEM. * and ** indicate $p<0.05$ and $p<0.005$ compared to control group, respectively.

## IV. DISCUSSIONS

*3*-Hydroxybenzaldehyde (*3*-HBA) is one of the three isomers of hydroxybenzaldehyde and a chemically synthesized water-soluble phenolic aldehyde[17]. Like salicylalehyde (2-hydroxybenzalhyde) that is another isomer of hydroxybenzaldehyde, *3*-HBA used as a chelating reagent[17], however, other function of *3*-HBA has remained unknown. On the other hand, protocatechuic aldehyde (*3, 4-di*hydroxybenzaldehyde; PCA) with a similar structure is known the efficacy in cardiovascular diseases through various studies[2,12,18-21]. Especially, PCA is capable to prevent the proliferation and migration of VSMCs and has capacity of as an anticoagulant[2].

In the present study, we demonstrated that 1) *3*-HBA prevents arteries from thickening by suppressing PDGF-induced VSMC proliferation and migration and 2) prevents thrombus formation. VSMCs show over 90% of viability even after 48 hours treatment in various dose of *3*-HBA (Figure 2). Within this time range, *3*-HBA showed its therapeutic effectiveness.

Vascular migration and proliferation are considered as key steps in the progression of atherosclerosis[1]. From the MTS experiments, we found that both *3*-HBA-pretreated groups for short and long term showed suppressing effect when cells were induced with PDGF (Figure 3**A**). Also, the upregulation of phosphorylation of AKT is a key modulator of cell proliferation[1]. In the present study, both the phosphorylated AKT and newly synthesized DNA labeled by BrdU were reduced after *3*-HBA was treated (Figure 3**B**, **C**).

In addition, VSMCs cell cycle inhibition is one of the therapeutic strategies for treatment of atherosclerosis[22]. Cell cycle progression is dependent on the expression and activation of cyclin-dependent kinase (CDK) with their regulatory coenzymes, the

cyclins[23]. In addition to interaction between CDK and cyclin complex, the E2F family of transcription factors with retinoblastoma protein controls expression of genes in quiescent condition[24]. In the present study, *3*-HBA blocked cells from entering synthetic (S), gap (G2), and mitotic (M) phase. We believe that *3*-HBA suppressed the expressions of cyclin D1 and Rb1 and regulated cells to enter quiescent (G0) phase. (Figure 4**A, B**).

Also, MMP-2, so called gelatinase A, leads VSMCs migrate and neointimal thickening. We investigated that *3*-HBA shows suppressing effect of VSMC migration by wound-healing experiments and MMP-2 expression.

Not only in VSMC migration and proliferation, but *3*-HBA showed anti-platelet effect in experimental animals. Thrombotic occlusion is the major cause of heart attack or stroke. For patients at risk of ischemic stroke, antithrombotics, such as heparin and aspirin, are used therapeutically. It is known that when heparin injected to the patients, it acts by binding to antithrombin III. In contrast, aspirin inhibits platelet aggregation by blocking thromboxane A2 synthesis[16,26]. When *3*-HBA introduced directly into whole blood, velocity of the platelet aggregation induced by ADP was decreased depending on the *3*-HBA concentration. Also, the velocity of aggregation whole blood collected from animals after *3*-HBA injection for a week had similar results to that of aspirin treated animals. Moreover, when tail thrombus induced by *κ*-carrageenan, the obstructed region of the tail was dissolved over time after *3*-HBA administration for a week (Figure 7). Further researches are needed to verify the underlying mechanism of *3*-HBA in attenuation of platelet aggregation.

In conclusion, *3*-HBA attenuated PDGF-induced VSMC proliferation and migration those are critical in pathophysiology of atherosclerosis by downregulating of the AKT phosphorylation and MMP-2 expression. Moreover, we observed that after administering

*3*-HBA, platelet aggregation was inhibited. These results provide that *3*-HBA is a potential candidate for the development of new therapeutic drug to prevent and treat atherosclerosis.

## V. CONCLUSION

These results support that *3*-hydroxybenzaldehyde affects in two different directions in atherosclerosis. *3*-hydroxybenzaldehyde acts as an inhibitor of both proliferation and migration of VSMCs that play an important role on pathophysiologic process of atherosclerosis. *3*-hydroxybenzaldehyde is responsible for inhibiting the activity of various enzymes and signal transmissions induced by PDGF in VSMC.

Furthermore, *3*-HBA shows the ability to reduce ADP-induced platelet aggregation in a dose-dependent manner and comparable effect of same dose of aspirin, well known as an antiplatelet. In addition, *3*-HBA has an excellent effect in restoring from κ-Carrageenan-induced thrombosis.

*3*-hydroxybenzaldehyde has a possibility as a therapeutic component on the atherosclerotic diseases.

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## 국문요약


### 죽상동맥경화에 대한 *3*-hydroxybenzaldehyde 의 효과

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임 수 정

본 연구는 기능이 알려지지 않았던 *3*-hydroxybenzaldehyde (*3*-HBA)가 혈관평활근세포에서 혈소판유래성장인자에 의한 증식 및 이동에 있어 저해제의 역할과 래트의 조건에 형성된 죽상동맥경화모델에서 항응고제의 역할을 통하여 신약개발의 후보로서의 가능성을 검증하는 것이다.

*3*-HBA 를 랫드의 혈관평활근세포에 전처리하였을 경우 혈소판유래성장인자가 유도되어도 세포의 성장주기 및 분열, 이동이 억제되었다. 이에 관련된 분자생물학적 변화양상은 Western blot 및 PCR 을 통하여 확인하였다. 또한 직접 실험동물로부터 획득한 전혈에 *3*-HBA 를 도입하거나, 7 일간 직접 동물에 *3*-HBA 를 투약하여 전혈을 채취한 경우 모두 ADP 로 혈전응고를 유도하였을 때 혈전 형성 속도를 감소시키는 효과를 보였다. 뿐만 아니라 7 일간 *3*-HBA 를 투약한 실험동물의 꼬리에 혈전을 형성하였을 경우, 형성된 혈전이 완화됨을 확인하였다.

결론적으로 본 연구를 통하여 물질 *3*-HBA 가 죽상동맥경화의 병리학적 양상인 혈관평활근세포의 증식 및 이동을 억제함과 혈전형성의 억제를 통하여 죽상동맥경화의 예방약물로의 가능성이 있다는 것을 시사한다.



핵심되는 말 : 죽상동맥경화, 혈전용해제, 혈관평활근세포, *3*-HBA. , *3*-Hydroxybenzaldehyde

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