Alagebrium chloride, a novel AGE cross linkage breaker, inhibits neointimal proliferation after carotid balloon injury in diabetic animal model

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Directed by Professor Moon-Hyoung Lee

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Dec. 2007

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Alagebrium chloride, a novel AGE cross linkage breaker, inhibits neointimal proliferation after carotid balloon injury in diabetic animal model

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Recent report has shown that longstanding hyperglycemia in diabetes leads to generation of AGEs (advanced glycation end products) and their accumulation induce vessel wall memory and vascular perturbation that lead to progression of atherosclerosis, plaque instability. Moreover, AGEs are reported to have a role in neointimal formation in animal experiment of arterial stenosis. AGE mediated cross link formation of proteins such as collagen will not only result in stiffening of the arteries, but will result in

increased extracellular matrix formation and increased vascular inflammation resulting in higher risk of neointimal proliferation. The in vitro study revealed a dose-dependently inhibitory effect of Alagebrium on AGE stimulated proliferation of RASMC. There was a significant dose dependent inhibition of AGE mediated reactive oxygen species formation by alagebrium. This was associated with a dose dependent inhibition of pMAPK formation as well. This cellular mechanism was involved in the regulatory effect of alagebrium on AGE-induced CTGF and ECM expression in RASMC. The in vivo study revealed a significant inhibition of neointima hyperplasia in ballooned injured rats treated with Alagebrium compared to placebo. In conclusion, alagebrium inhibits neointimal hyperplasia after carotid balloon injury because it blocks the intracellular ROS synthesis resulting in inhibition of vascular smooth muscle cell proliferation. The inhibitory effect of alagebrium on neointimal formation may be also mediated by reduction of extracellular matrix production.

Key Words : AGE, crosslink breaker, neointimal hyperplasia.

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I. INTRODUCTION

Restenosis remains a significant limitation for percutaneous coronary intervention (PCI) affecting up to 30% of patients treated with this procedure and the restenosis rate is even higher in diabetes[1]. It is assumed to be the result of neointimal formation characterized by an inflammatory reaction at

the site of injury, migration and proliferation of vascular smooth muscle cells and the synthesis of excessive extracellular matrix[2]. However, exact pathogenic mechanisms leading to the exaggerated restenosis in diabetes are poorly understood.

A recent report has shown that longstanding hyperglycemia leads to generation of AGEs (advanced glycation end products), the products of nonenzymatic glycation/oxidation of proteins/lipids, and their accumulation induce vessel wall memory and vascular perturbation that lead to progression of atherosclerosis, plaque instability[3-8]. Moreover, AGEs are reported to have a role in neointimal formation in animal experiment of arterial stenosis[9-11]. In these reports, receptor for AGEs/ligand interaction plays a key role in neointimal formation after vascular injury irrespective of diabetes status and these findings suggest a novel target to minimize neointimal hyperplasia[10-12]. Recent report showed that hyperglycemia leads to generation of AGEs and accumulation of AGEs and their interaction with the receptor for AGE (RAGE), involves the plausible mechanism of induction of the vessel wall memory and sustained perturbation[13-15]. These processes lead to progression of atherosclerosis, plaque instability, and the emergence of clinical events. AGEs are generated via polyol pathway. In this pathway, glucose is reduced

to sorbitol by aldose reductase (AR); fructose generated by this pathway is converted into fructose-3-phosphate by the activation of 3-phosphokinase. This leads to the generation of 3-deoxyglucosone, a central precursor in the generation of an array of AGEs, in particular, carboxymethyl-lysine (CML)-adducts and others. AGE mediated cross link formation of proteins such as collagen will not only result in stiffening of the arteries, but will result in increased extracellular matrix formation and increased vascular inflammation resulting in increased risk of neointimal proliferation[4, 16-18]. Increased AGE cross linkage will result in activation of prosclerotic cytokines such as TGF-β1 which will result in increased proliferation of extracellular matrix. Also, AGE accumulation and cross linkage formation is associated with increased proinflammatory cytokines and expression of the receptor of AGE(RAGE), resulting in increased vascular inflammation[17-19].

Therefore, in this study, we hypothesize that Alagebrium chloride (ALT-711), an AGE cross link breaker, will inhibit neointimal proliferation through above mentioned mechanisms in a streptozocin induced diabetic rat carotid artery balloon injury model.

II. MATERIALS AND METHODS

1. Animal experiments

20 eight-week old male, Sprague-Dawley rats weighing 200 to 250g received streptozocin at 50 mg/kg intraperitoneally. The diabetic rats received long acting insulin (Lantus, Aventis Pharma Deutschland GmbH, Germany) 2 unit/day after the streptozocin injection. Sixteen weeks later, the rats were randomized into two study groups: 10 diabetic rats receiving placebo and 10 diabetic rats receiving Alagebrium 10 mg/kg. The Alagebrium chloride was mixed with pulverized standard chow to a final concentration of 0.015% (wt/wt) and treated for 4 weeks. After 4 weeks, the carotid artery balloon injury was performed. The control rats were also divided into two groups: 10 non diabetic rats administered with placebo, 10 non diabetic rats receiving Alagebrium 10mg/kg. After 4 weeks of administration, carotid artery balloon injury was performed.

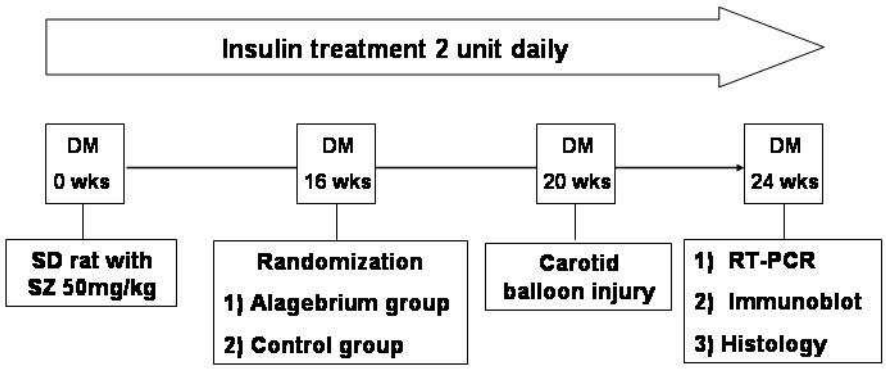


Figure 1. Schematic representation of experimental animal model

2. Isolation and culture of rat aortic vascular smooth muscle cells

Rat aortic VSMCs were isolated as previously described. The thoracic aortas from 6- to 8-week-old rats were removed and transferred in serum-free Dulbecco's modified Eagle' medium (DMEM; Invitrogen Co, Carlsbad, CA, USA) containing 100 units/㎖ of penicillin and 100 ㎍/㎖ of streptomycin. The aorta was freed from connective tissue, transferred into Petri dish containing 5 ㎖ of an enzyme dissociation mixture containing DMEM with 1 ㎎/㎖ of collagenase type I (Sigma, St. Louis, MO, USA) and 0.5 ㎍/㎖ elastase (USB Bioscience, Cleveland, OH, USA), and incubated for 30 min

and at 37°C. The aorta was the transferred into DMEM and the adventitia was stripped off with forceps under a binocular microscope. The aorta was transferred into a plastic tube containing 5 ㎖ of the enzyme dissociation mixture and incubated for 2 h at 37°C. The suspension was centrifuged (1,500 rpm for 10 min) and the pellet was resuspended in DMEM with 10% fetal bovine serum (FBS). Rat aortic VSMCs were cultured in DMEM supplement with 10% FBS, 100 IU/㎖ penicillin, 100 ㎍/㎖ streptomycin in 75-㎠ flasks at 37°C in a humidified atmosphere of 95% air and 5% CO_2 (Forma Scientific, Inc., Marjetta, OH, USA.).

3. Cell proliferation assay

Rat aortic VSMCs were plated in triplicate wells of 96 well plates at a density of 1 x 10^4 per well. Pretreatment with alagebrium was done 3 hrs prior to exposure to AGE (50 μg/mL) for 3 hrs. After cell treatment, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (Sigma, St. Louis, MO, USA) was added to each well to a final concentration of 0.5 mg/mL and was incubated at 37℃ for 3 hr to allow MTT reduction. The formazan crystals were dissolved by adding dimethylsulfoxide (DMSO) and absorbance was measured at the 570 nm with a spectrophotometer.

Experiments were performed in triplicate.

4. Measurement of intracellular ROS generation

Rat aortic VSMCs are labeled with 2′, 7′-dichlorodihydrofluorescein diacetate (H_2DCFDA; Molecular Probe, CA, USA). The probe H_2DCFDA (10 μM) enters the cell and the acetate group on H_2DCFDA is cleaved by cellular esterases, trapping the non-fluorescent 2′, 7′-dichlorofluorescin (DCFH) inside. Subsequent oxidation by reactive oxygen species yields the fluorescent product DCF. The dye, when exposed to an excitation wavelength of 480 nm, emits light at 535 nm only when it has been oxidized. Labeled rat aortic smooth muscle cells are examined using a luminescence spectrophotometer for oxidized dye. The quiescent cells were treated with alagebrium or/and AGE before labeling with H_2DCFDA.

5. RT-PCR analysis

The expression levels of various proteins were analyzed by reverse transcription polymerase chain reaction (RT-PCR). Total RNA was prepared by the Ultraspect^{TM}-II RNA system (Biotecx Laboratories, Inc., USA) and single-stranded cDNA was then synthesized from isolated total RNA by Avian

Myeloblastosis virus (AMV) reverse transcriptase. A 20 µl reverse transcription reaction mixture containing 1 µg of total RNA, 1X reverse transcription buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100), 1 mM deoxynucleoside triphosphates (dNTPs) 0.5 unit of RNase inhibitor, 0.5 µg of oligo(dT)15 and15 units of AMV reverse transcriptase was incubated at 42℃ for 15 min, heated to 99℃ for 5 min, and then incubated at 0-5℃ for 5 min. PCRs were performed for 35 cycles with 3' and 5' primers based on the sequences of various genes. Primers were as follows: RAGE: 5'-GGCCTTCCTCGGCGCAGACC-3', 5'-TAGATGCCCTCATCCTCATGC-3' (260 bp); COX-2: 5'-TCCAATCGCTGTACAAGCAG-3', 5'-TCCCCAAAGATAGCATCTGC-3' (230 bp); Collagen type III: 5'-AGATGCTGGTGCTGAGAAG-3', 5'-TGGAAAGAAGTCTGAGGAAGG-3' (312 bp); Fibronectin 5'-GTGAAGAACGAGGAGGATGTG-3', 5'-GTGATGGCGGATGATGTAGC-3' (300 bp); Connective tissue growth factor: 5'-AAGAAGACTCAGCCAGACC-3', 5'-AGAGGAGGAGCACCAAGG-3' (235 bp). GAPDH (primers 5'-ACCACAGTCCATGCCATCAC-3' and 5'-TCCACCACCCTGTTGCTGTA-3' (450 bp)) was used as the internal standard. The signal intensity of the amplification product was normalized to its respective GAPDH signal

intensity.

6. Immunoblot analysis

Rat aortic VSMCs were treated with Alagebrium before the addition of advanced glycation endproduct as mitogenic stimulus. Cells were washed once in PBS and lysed in a lysis buffer (Cell signaling, Beverly, MA, USA) containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 mg/ml leupeptin and 1 mM PMSF. Protein concentrations were determined using the Bradford protein assay kit (BioRad, Hercules, CA, USA). Proteins were separated in a 12% SDS-polyacrylamide gel and transferred to PVDF membrane (Millipore Co, Bedford, MA, USA). After blocking the membrane with Tris-buffered saline-Tween 20 (TBS-T, 0.1% Tween 20) containing 5% non-fat dried milk for 1 hr at room temperature, membranes were washed twice with TBS-T and incubated with primary antibodies for 1 hr at room temperature or for overnight at 4°C. The following primary antibodies were used: rabbit anti-ERK, mouse anti-phospho ERK (Santa Cruz Biotechnology, CA, USA). The membrane were washed three times with TBS-T for 10 min, and then incubated for 1 hr at room temperature

with horseradish peroxidase (HRP)-conjugated secondary antibodies. After extensive washing, the bands were detected by enhanced chemiluminescence (ECL) reagent (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The band intensities were quantified using a Photo-Image System (Molecular Dynamics, Uppsala, Sweden).

7. Carotid artery balloon injury model

The animals were anesthetized intraperitoneally with ketamine (80mg/kg). After anesthesia, the left carotid artery was exposed by cervical midline excision. The proximal portion of the left common carotid artery and the origin of the left internal carotid artery were temporarily ligated with surgical threads to prevent excessive bleeding during the balloon catheter insertion. Part of the external carotid artery wall was excised after which a 2 Fr Fogarty balloon catheter (Baxter Healthcare Corp, Irvine, CA, USA) was inserted. After the insertion of the balloon catheter, the temporarily ligated proximal left common carotid artery was untied and the Fogarty catheter was advanced towards the direction of the ascending aorta. The balloon was inflated just enough to develop a mild resistance against the arterial wall (0.2ml). The expanded balloon catheter was than pulled back to inflict uniform injury along

the entire common carotid artery wall. This procedure was repeated 3 times for complete denudation of the intima after which the Fogarty balloon catheter was removed and the proximal external carotid artery was ligated. Sham operations were performed on the right common carotid arteries.

8. Histology and Immunohistochemistry

SD rats treated with ballon injury were killed and their carotid artery excised. The vessels were perfusion-fixed with 10% (v/v) neutral buffered formaldehyde for 24h, transversely sectioned into serial thick sections, and embedded in paraffin by routine methods. Sections of 2 μm-in thickness were mounted on a gelatin-coated glass slides to ensure different stains could be used on successive sections of tissue cut through the areas of ballon injury. After deparaffinization and rehydration, the sections were analyzed with mouse anti-RAGE obtained from Santa Cruz Biotechnology Inc (CA, USA), rabbit anti-collagen type I, and rabbit anti-collagen type III from Cell Signaling Technology (MA, USA). Fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG and Texas red-conjugated goat anti-mouse IgG or mouse anti-goat IgG from Jackson ImmunoResearch Lab. (PA, USA) were used as secondary antibody. All images were made by using an

excitation filter under reflected light fluorescence microscopy and transferred to a computer equipped with MetaMorph software ver. 4.6 (Universal Imaging Corp.).

9. Statistical analysis

Data are presented as mean ± S.E.M. of more than three separate experiments performed in triplicate. Where results of blots and RT-PCR are shown, a representative experiment is depicted. Comparisons between multiple groups were performed with one-way ANOVA (Analysis of Variance) with Bonferroni's test. Statistical significance was defined as $p< 0.05$ and $p< 0.01$.

III. RESULTS

1. Antiproliferative effect of alagebrium in AGE-stimulated rat aortic VSMCs

Advanced glycation end product (AGE) formation may contribute to the progression of atherosclerosis, particularly in diabetes. AGE could pass through the cell membrane via receptor for AGE (RAGE) and stimulate MAP kinases to cause the proliferation. In order to investigate whether alagebrium might regulate the proliferation of rat aortic VSMCs stimulated by AGE, cells were pretreated with various concentrations (0 - 100 μM) of alagebrium for 3 hrs prior to exposure of AGE (50 μg/ml). Alagebrium inhibited AGE-stimulated proliferation (Figure 2). Namely, this finding shows alagebrium has an antiproliferative effect on rat aortic VSMCs stimulated by AGE.

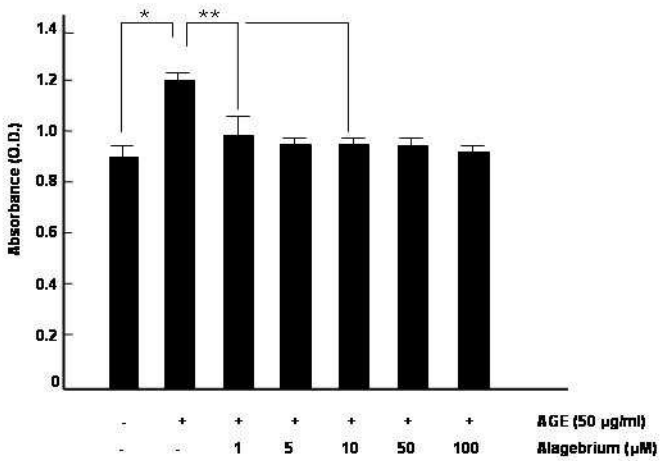


Figure 2. Antiproliferative effect of alagebrium in AGE-stimulated rat aortic vascular smooth muscle cells. Alagebrium inhibits proliferation of VSMCs in dose dependent manners (*P<0.01, ** P<0.05).

2. Effect of AGE and/or alagebrium on the formation of intracellular ROS in rat aortic VSMCs

Intracellular reactive oxygen species can stimulate MAP kinases to cause the proliferation of different cells. To investigate the effect of AGE on the formation of intracellular reactive oxygen species, cells were treated with various concentrations of AGE. Following 3 hours incubation of AGE, the ROS production of rat aortic VSMCs was increased dose dependently,

indicating that AGE could be one of growth stimulators of rat aortic VSMCs (Figure 3). In order to investigate whether alagebrium might modify the effect of AGE on the ROS production of rat aortic VSMCs, cells were pretreated with various concentrations of alagebrium for 30 min prior to exposure of AGE (50 μg/ml). Figure 4 showed that alagebrium reduced the formation of reactive oxygen species generated in a dose-dependent manner.

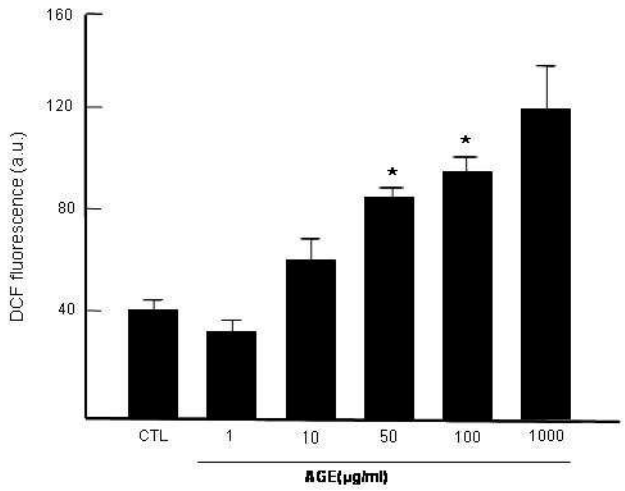


Figure 3. Effect of AGE on the formation of intracellular ROS in rat aortic VSMCs. AGE stimulated growth of rat aortic VSMCs in dose dependant manners

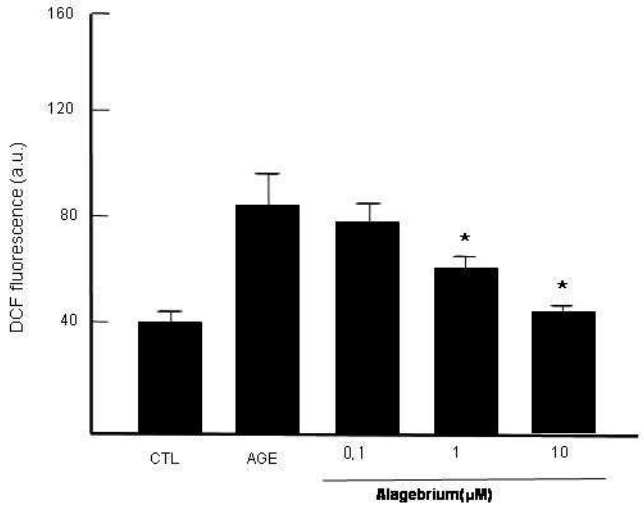


Figure 4. Effect of alagebrium on the formation of intracellular ROS in rat aortic VSMCs treated with AGE. alagebrium reduced the formation of reactive oxygen species generated in a dose-dependent manner.

3. Effect of alagebrium on activation of ERK and COX-2 mRNA expression stimulated by AGE

In the mechanisms of cellular survival and proliferation, the activation of ERKs plays an important role in gene regulation. ERK is one of dual specificity kinases in MAPKs. Phosphorylation of ERKs (42 and 44 kDa) was detected by immunoblot assay. It has been known that AGE stimulates the activation of ERK MAPK in rat aortic VSMCs. Thus, we investigated the effect of alagebrium on ERK MAPK activation in AGE-stimulated rat aortic

VSMCs. We demonstrated that ERK MAPK activation was seen within 30 min with 50 μg/ml of AGE. These signaling activation was significantly inhibited by pretreatment with 1 and 10 μM of alagebrium (Fig 5).

Because COX-2 expression is associated with ROS production and ERK activation, and the proliferation of rat aortic VSMCs is mediated by COX-2 expression, the effect of alagebrium on the expression of COX-2 in the AGE-stimulated rat aortic VSMCs was examined. COX-2 mRNA expression increased after incubation with 50 μg/ml of AGE. But alagebrium significantly inhibited expression of COX-2 mRNA in AGE-stimulated rat aortic VSMCs (Fig 6).

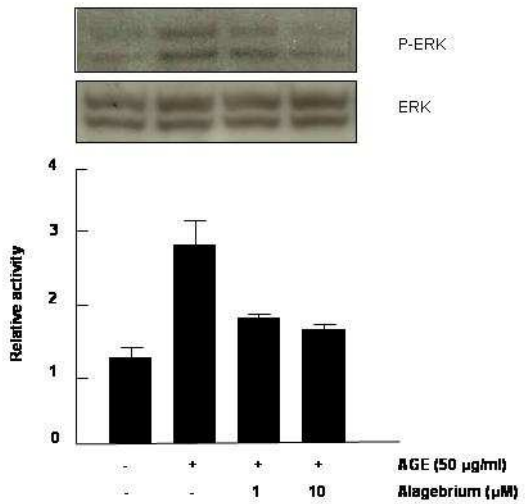


Figure 5. Effect of alagebrium on activation of ERK mRNA expression

stimulated by AGE. ERK MAPK activation was seen within 30 min with 50 μg/ml of AGE. These signaling activation was significantly inhibited by pretreatment with 1 and 10 μM of alagebrium

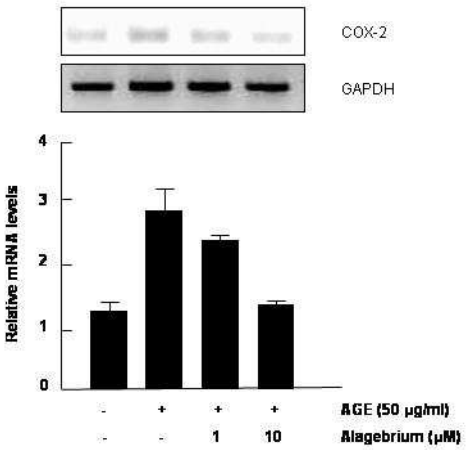


Figure 6. Effect of alagebrium on activation of COX-2 mRNA expression stimulated by AGE. COX-2 mRNA expression increased after incubation with 50 μg/ml of AGE. But alagebrium significantly inhibited expression of COX-2 mRNA in AGE-stimulated rat aortic VSMCs.

4. Inhibitory effects of alagebrium on expression of CTGF and extracellular matrix in AGE-stimulated RASMCs

To demonstrate whether a direct cellular mechanism was involved in the regulatory effect of alagebrium on AGE-induced CTGF and ECM expression in rat aortic VSMCs, we performed experiments in cultured rat aortic VSMCs.

As illustrated in Figure 7, AGE (50 μg/ml) treatment for 24 h markedly increased the mRNA expression of Collagen type III (Col III), Fibronectin (FN) and connective tissue growth factor (CTGF) in rat aortic VSMCs. Alagebrium (1and 10 μM) dose-dependently abolished this effect (Fig. 7).

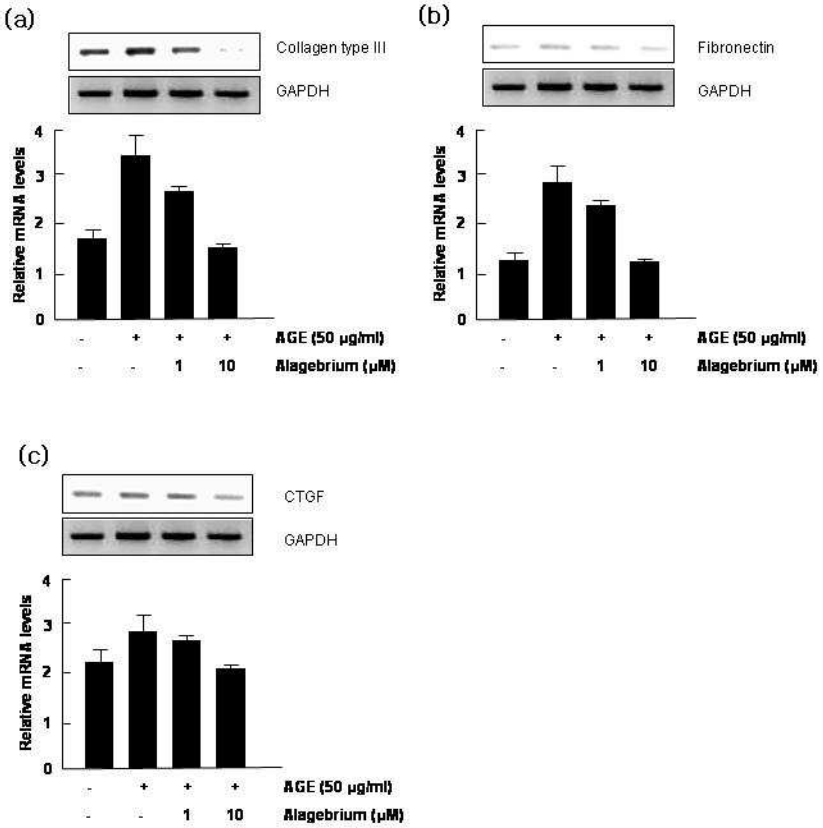
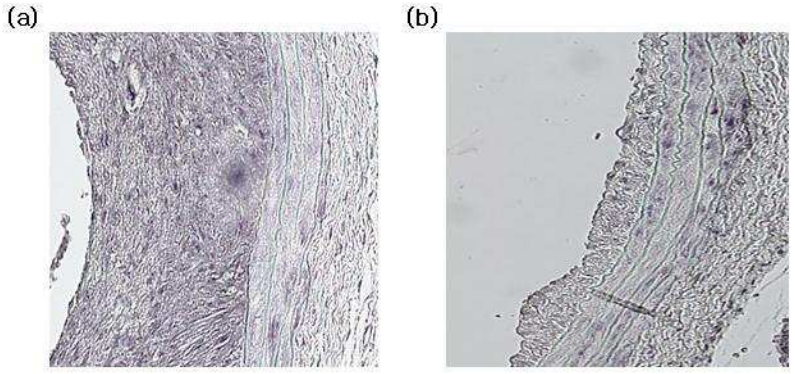


Figure 7. Effects of alagebrium on expression of CTGF and extracellular matrix in AGE-stimulated RASMCs AGE (50 μg/ml) treatment for 24 h markedly increased the mRNA expression of Col III (a), FN (b) and CTGF (c)

in rat aortic VSMCs. Alagebrium (1and 10 μM) dose-dependently abolished this effect.

5. Effect of alagebrium on the expression of RAGE in VSMCs

To demonstrate whether RAGE expression was involved in the regulatory effect of alagebrium on AGE-induced cellular process in rat aortic VSMCs, we performed experiments in cultured rat aortic VSMCs. AGE (50 μg/ml) treatment for 24 h markedly increased the mRNA expression of RAGE in rat aortic VSMCs. Alagebrium (1and 10 μM) dose-dependently abolished this effect. This effect was also demonstrated by immunohistochemistry. (Fig. 8)



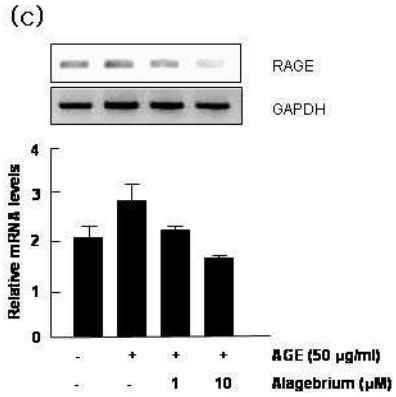


Figure 8. Effect of alagebrium on the expression of RAGE in VSMCs
(a) and (b) RAGE expression was significantly decreased with alagebium treatment in vivo, comfirmed by immunohistochemistry. (c) in cultured media, AGE increased expression of mRNA of RAGE. This effect was significantly attenuated by alagebrium.

6. Extracellular matrix and neointima formation by alagebrium in balloon-injury of STZ-rats

To test the role of alagebrium in neointima response after balloon injury, daily supply of alagebrium were used. Withdrawal of the inflated balloon resulted in vascular injury leading to neointima formation. Seven days after injury, the inner vessel surface was covered with one or several layers of ovoid, irregular-shaped cells forming the neointima. In the present study, neointima formation in balloon-injured arteries was confirmed by histology. Figure 9

shows that the density of neointima formation after balloon injury was much thicker than sham. However, no further neointimal growth was observed at STZ-rat treated with alagebrium after balloon injury. At this time, protein expression composed of extracellular matrix in STZ-rat treated with alagebrium was reduced more than sham.

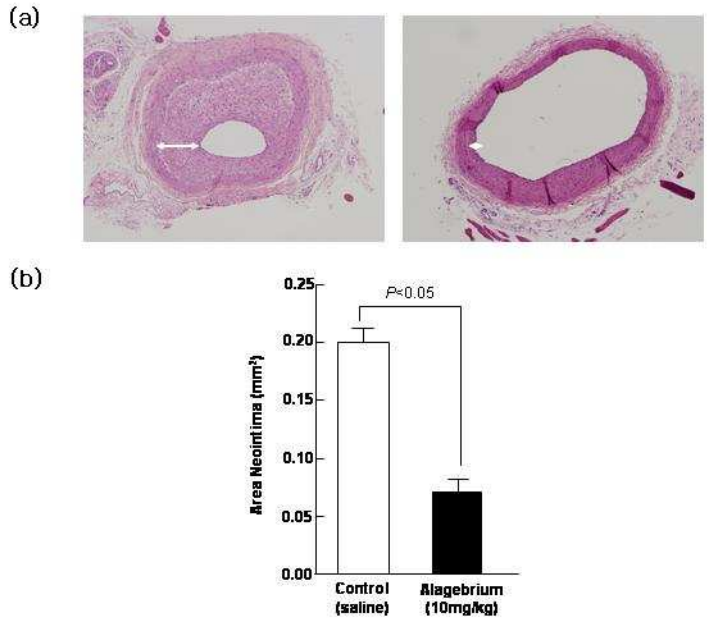


Figure 9. The effect of alagebrium on extracellular matrix and neointima formation in balloon-injury of STZ-rats (a) neointima hyperplasia was decreased by alagebrium treatment in diabetic rat (H-E stain) (b) extracellular matrix in STZ-rat treated with alagebrium was significantly reduced more than sham. *(P<0.05)*

IV. DISCUSSION

Our present study demonstrates that alagebrium chloride (ALT-711), an AGE cross link breaker, inhibits neointimal proliferation in a streptozocin induced diabetic rat carotid artery balloon injury model. This is the first report that alagebrium can directly inhibit the pro-atherosclerotic effect of AGE on RASMCs. These effects of alagebrium were mediated by inhibition of ERK signaling pathway and down-regulation of COX-2 mRNA expression via reduction of intracellular ROS induced by AGE. Furthermore, we found that these anti-proliferative of alagebrium was resulted from inhibition of RAGE expression.

Macrovascular complications develop in >50% of the diabetic population and account for 50–60% of the mortality in this high-risk population[1]. Dyslipidemia, hypertension, and hyperglycemia all play a role in the development of diabetes-associated atherosclerosis, although the specific contributions from each of these independent risk factors remain controversial. Previous studies that used exogenous administration of AGEs to mimic diabetic serum concentrations indicated that AGEs could induce atherosclerosis[6-8]. Furthermore, AGEs interact with endothelial cells to induce the expression of atherogenic adhesion molecules implicated in atherogenesis.

Physical glycation is a major source of ROS and reactive carbonyl species

(RCS) that are generated by both oxidative (glycoxidative) and nonoxidative pathways. In addition, glucose itself can auto-oxidize to accelerate the formation of AGEs, leading to oxidative damage. AGEs are known to accumulate in various tissues at accelerated rates in the diabetic condition and are implicated in the development of diabetic vascular complications. In vitro work has shown that ligation of the advanced glycation end products receptor (RAGE) is part of the complex interactions within oxidative stress and vascular damage, particularly in atherosclerosis and in the accelerated vascular damage that occurs in diabetes.

The broad consequences of the RAGE–ligand interaction for cellular properties are emphasized by the spectrum of signaling mechanisms. One such consequence of this interaction can lead to the generation of ROS. Other consequences include the production of growth factors and cytokines, chronic inflammatory responses, and cellular and vascular dysfunction associated with diabetic complications. However, the treatment with alagebrium suppressed not only the ROS formation but also RAGE expression induced by AGE in diabetic condition (Figure 3, 4, and 8). Moreover, the proliferation of VSMCs was reduced by alagebrium via blocking the activation of ERKs (Figure 2 and 5)

Studies with inflammatory cells such as human monocytes have demonstrated that stimulated diabetic conditions in vitro such as high glucose (HG) culture conditions or treatment with AGEs lead to oxidative stress, and further induce pro-inflammatory cytokines and related genes via activation of specific signaling pathways and transcription factors. Further evidence for the role of AGEs in diabetes, showing that AGEs can augment inflammatory reponses by up-regulating COX-2 via RAGE and multiple signaling pathways, thereby lead to monocyte activation and vascular cell dysfunction. As shown in Figure 6, the expression of COX-2 was enhanced in AGE-stimulated VSMCs but sharply decreased dose-dependently by the treatment with alagebrium.

Connective tissue growth factor (CTGF) is a potent profibrotic factor implicated in pathologic fibrosis processes, including skin disorder, tumor development, lung fibrosis, and renal disease. In cardiovascular system, CTGF is overexpressed in the atherosclerotic lesions, myocardium of infracted rats and patients with cardiac ischemia, and arteries of hypertensive animals. Moreover, CTGF and extracellular matrix (ECM) regulates cell proliferation and apoptosis, migration and fibrosis in VSMCs. In this study, AGE stimulated the expression of CTGF and ECM compared with normal

control but the expression of CTGF and ECM genes was reduced after treatment with alagebrium. (Figure 7)

Studies in experimental obese Zucker rats, a model of insulin resistance with the AGE formation inhibitor pyridoxamine, have shown marked attenuation of the characteristic hyperlipidemia, reduced ALEs, and hypertension, indicating interactions between AGEs and lipids[19]. One of the seminal in vivo studies performed in experimental diabetes-associated atherosclerosis demonstrated attenuation of plaque formation with the soluble receptor for AGEs (sRAGE)[18].

More recently, therapeutic approach has involved the use of a thiazolium derivative alagebrium, a compound that is suggested to be capable of breaking AGE cross-links, thereby removing preformed AGEs[3-5]. Alagebrium has proved successful as an intervention therapy in established diabetic microvascular complications and has also been reported to improve vascular compliance in aging humans. Other studies in the diabetic context have shown that alagebrium attenuates both cardiac and renal accumulation of AGEs in association with benefits on renal and cardiac injury[8, 12-14]. Furthermore, the present study showed the inhibitory effects of alagebrium on rat aortic VSMCs proliferation at in vitro study and neointimal formation at in vivo

study through the reduction of ERK activation and COX-2 expression. The inhibitory mechanism of alagebrium was involved in the inhibition of intracellular ROS synthesis.

In conclusion, alagebrium inhibits neointimal hyperplasia after carotid balloon injury because it blocks the intracellular ROS synthesis resulting in inhibition of vascular smooth muscle cell proliferation. The inhibitory effect of alagebrium on neointimal formation may be also mediated by reduction of extracellular matrix production.

V. CONCLUSION

Alagebrium treatment in diabetic rat inhibits neointimal hyperplasia after carotid balloon injury because it blocks the intracellular ROS synthesis resulting in inhibition of vascular smooth muscle cell proliferation. The inhibitory effect of alagebrium on neointimal formation may be also mediated by reduction of extracellular matrix production. Furthermore, this effects on extracellular matrix and neointima formation were also mediated by decreased RAGE expression on rat vascular smooth musle cells.

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ABSTRACT (KOREAN)

당뇨 백서 모델의 경동맥 손상후 신생 내막 증식에 대한 AGE 가교체 결합 분해제인 alagebrium의 효과

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최근 보고에 의하면 당뇨에서의 지속되는 고혈당증은 AGEs 의 생성을 야기하며 그것들의 축적은 죽상동맥경화증과 플라크 불안정의 진행을 이끄는 혈관벽 기억과 손상을 유도한다. 게다가, 동맥협착증 동물 실험에서 AGEs 의 신생혈관내막 형성 역할이 보고 되고 있다. 콜라겐과 같은 단백질 교차결합 형성에 관계하는 AGE 는 동맥들을 경직시킬 뿐만 아니라 세포내막 형성 상승과 신생혈관 내막 증식의 높은 위험성을 가진 혈관 염증을 증가시키기도 한다. In vitro

연구에서는 AGE 처리된 RASMC 의 증식에서 alagebrium 의 농도 의존적인 억제 효과를 나타냈다. 이는 활성산소종 형성에 관계된 AGE 에 대해 alagebrium 의 의미 있는 농도 의존성 억제가 나타났다. 이는 또한 pMAPK 형성의 농도 의존적 억제와 연관됐다. 이러한 세포의 기작은 RASMC 에서 AGE 가 유도된 CTGF 와 세포 외 기질 발현에 영향을 주는 alagebrium 과 연관되었다. In vivo 연구에서는 플라시보와 비교하여 alagebrium 을 처리한 풍선도관으로 손상된 쥐에서 신생 혈관내막 과형성의 억제가 일어났다. 결과적으로, alagebrium 은 혈관 평활근 세포 증식의 억제를 일으키는 세포 내 활성산소종을 제어하기 때문에 경동맥 풍선도관 손상 후 신생 혈관내막 과형성을 억제하였다. 신생혈관 형성에 영향을 주는 alagebrium 의 억제 효과는 세포 외 기질 형성을 감소시키는데 연관되어 있을 것이다.

핵심되는 말 : AGE, 교체 결합 분해, 신생 내막 증식