Angiotensin II type 1 receptor blocker losartan attenuates bioprosthetic valve leaflet calcification in a rabbit intravascular implant model

Hong Ju Shin

Department of Medicine

The Graduate School, Yonsei University

Angiotensin II type 1 receptor blocker losartan attenuates bioprosthetic valve leaflet calcification in a rabbit intravascular implant model

Directed by Professor Young Hwan Park

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

Hong Ju Shin

June 2016

This certifies that the Doctoral Dissertation of Hong Ju Shin is approved.

Thesis Supervisor: Young Hwan Park

Thesis Committee Member: Han Ki Park

Thesis Committee Member: Jong-Chul Park

Thesis Committee Member: Lucia Kim

Thesis Committee Member: Wan Namkung

The Graduate School
Yonsei University

June 2016

ACKNOWLEDGEMENTS

First of all, I would like to show my respect and thank Professor Young Hwan Park, not only for directing me as a student but also giving me opportunities to be a better congenital cardiac surgeon. I would also like to thank Doctor Dae-Hyun Kim for helping me perform the experiments.

I thank my parents and parents-in-law for supporting me all the time. I would like to thank my wife, Yoo Sun Shim for being next to me all the time. I would like to mention my three children (Won Jun, Yeon Jae, Yoon Woo) and I would like to say I love you all.

Hong Ju Shin

<TABLE OF CONTENTS>

LIST OF FIGURES

ABSTRACT

Angiotensin II type 1 receptor blocker losartan attenuates bioprosthetic valve leaflet calcification in a rabbit intravascular implant model

Hong Ju Shin

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Young Hwan Park)

There are some evidences that angiotensin II type I receptor blocker (ARB) could reduce the structural valve deterioration. However, the anticalcification effect on the bioprosthetic heart valve is not yet investigated. Thus, we investigated the effects of the ARB on calcification of implanted bovine pericardial tissue in a rabbit intravascular implant model. A total of 16 male New Zealand White rabbits (20 weeks old, body weight: 2.98~3.34 kg) were used in this study. Commercially available bioprosthetic heart valve leaflet of bovine pericardium was trimmed to a 3-mm triangle shape and implanted to both external jugular veins of the rabbit. The ARB group (n=8) was given 25 mg/kg of powdered losartan daily until 6 weeks after surgery by directly administration in buccal pouch of the animals. The control group (n=8) was

given 5 ml of normal saline with the same method. After 6 weeks, quantitative calcium determination, histological evaluation, and western blot analysis about interleukin-6, osteopontin, and BMP-2 were performed to identify the anti-calcification effect and its mechanisms of losartan. No deaths or complications such as infection or hematoma were recorded during the experiment. All animals were euthanized on the planned date. Calcium measurements level in the ARB group (2.28 ± 0.65 mg/g) was significantly lower than that in the control group (3.68 ± 1.00 mg/g) ($p = 0.0281$). Immunohistochemistry analyses revealed that bone morphogenetic protein 2 (BMP-2)-positive reactions were significantly attenuated in the ARB group. Western blot analysis showed that losartan suppressed the expression of interleukin-6, osteopontin, and BMP-2. Our results indicate that losartan significantly attenuates post-implant degenerative calcification of bovine pericardial bioprosthesis in a rabbit intravascular implant model. Further studies are required to assess the effects of ARBs on bioprosthetic heart valve tissue in orthotopic implantations using a large animal model.

Key words: bioprosthetic heart valve, degenerative calcification, rabbit, animal model, intravenous implantation

Angiotensin II type 1 receptor blocker losartan attenuates bioprosthetic valve leaflet calcification in a rabbit intravascular implant model

Hong Ju Shin

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Young Hwan Park)

I. INTRODUCTION

Bioprosthetic heart valve replacements fabricated from either glutaraldehyde-fixed porcine aortic valves or bovine pericardium are widely used for surgical therapy of end-stage heart valve disease.[1] However, most of these bioprostheses will eventually fail and require replacement, primarily due to structural valve deterioration from leaflet calcification.

Modern design techniques and anticalcification treatments applied to currently available bioprosthetic valves have greatly improved the durability of bioprostheses implanted into adult patients[2]; however, dystrophic calcification leading to early bioprosthetic valve failure is still a large problem in children and young adults.[3] Actual long-term durability of bioprosthetic pulmonary

valves implanted into children and young adults is not well defined because of the small number of patients and the relatively short duration of follow-up in previous studies.[4] Bioprosthetic leaflet calcification occurs by complex mechanisms that involve interactions between hosts and implant factors. The factors influencing bioprosthetic valve and other biomaterial mineralization include: (1) host metabolism, (2) implant structure and chemistry, and (3) mechanical factors.[1] Three generic strategies have been investigated for preventing calcification of biomaterial implants: (1) systemic therapy with anticalcification agents[5]; (2) local therapy with implantable drug delivery devices[6]; and (3) biomaterial modifications such as removal of a calcifiable component, addition of an exogenous agent, or chemical alteration.[7,8] However, none of these therapies have shown superior results.

Blockade of the renin-angiotensin-aldosterone system has been suggested recently as a potential therapy for aortic valve sclerosis and arterial calcification.[9-13] Angiotensin receptor blockade can inhibit arterial calcification by disrupting vascular osteogenesis. This suggests that that angiotensin II (Ang II) type 1 receptor blocker (ARB) may be a novel treatment option for patients suffering from vascular calcification.[12] O'Brien et al.[14] documented the presence of angiotensin converting enzyme (ACE) and its enzymatic product, Ang II, in aortic valve stenosis (AS) lesions. Of particular interest, one retrospective clinical study demonstrated that ACE inhibitors were associated with reduced mineralization within AS valves.[9] Some reports suggest Ang II may have a role in aortic valve calcification.1[5, 16] Interleukin-6 (IL-6) activity increases bone morphogenic protein 2/4 (BMP-2/4) expression in vessels and valve tissue, and

this is a major cause of vascular calcification.[17] A recent study reported that ARB reduced valve degeneration by suppressing the production of cytokines such as interleukin-6, which mediate inflammatory responses.[18, 19] However, there are few studies of inflammatory cytokines in xenogenic tissue such as bovine pericardium, and it is unknown if inflammatory cytokine suppression could prevent bioprosthetic valve degeneration. We hypothesized that ARB could suppress the expression of inflammatory cytokines in vascular tissue of bovine pericardial xenografts, thereby retarding the valve degenerative or suppressing calcification. Here, we investigated the effects of the ARB losartan on bioprosthetic valve calcification using previously reported our rabbit intravenous implantation model.[20]

II. MATERIALS AND METHODS

1. Implant preparation

Commercially available bovine pericardial bioprosthetic heart valves (BHVs) (Carpentier-Edwards Perimount Magna pericardial prosthesis; Edwards Lifesciences, Irvine, CA, USA) were used in the study. Before implantation, BHVs were rinsed for 30 min in 500 ml sterile physiological saline solution. Each leaflet was incised along the frame of the BHV using a no. 11 surgical blade, and then placed into another rinsing basin filled with sterile saline. The leaflet was trimmed with Metzenbaum scissors just before implantation.

2. Anesthesia, surgical procedure, and postoperative care

Sixteen healthy New Zealand White rabbits (20 weeks old; mean body weight 3.17 ± 0.10 kg, range 2.98□3.34 kg) were used in this study. Each animal was injected intramuscularly with 5 mg/kg xylazine and 10 mg/kg Zoletil® as a premedication. After intubation with a 3.0 mm endotracheal tube, isoflurane was used to maintain inhalation anesthesia. All animals received normal saline (10 ml/kg/h) throughout the surgical procedure. Surgical procedures were the same as those reported previously.[20]

All surgical procedures were performed with a 3.5 magnification surgical loupe. After placing the animal in a dorsal recumbent position, a ventral cervical midline incision was made. The left external jugular vein was exposed by

dissecting the sternohyoid muscles, at which time 50 IU/kg heparin was injected intravenously.

For patch implantation, the left jugular vein was temporarily occluded with a 4-0 silk and 5 fr feeding tube. Then, a longitudinal incision of approximately 8 mm length was made using a no. 11 surgical blade. For the intravenous implant, a 3-mm triangle-shaped BHV leaflet was fixed to the internal wall of the vein using two 8-0 polypropylene sutures (Figure 1A). To reduce the possibility of vessel occlusion due to lumenal narrowing, patch angioplasty also was performed at the incision site with an approximately 4 mm x 8 mm diamond-shaped BHV leaflet (Figure 1B). The completed procedures are depicted in Figure 1C and D. The right jugular vein also was implanted with a BHV leaflet using the same method. The subcutaneous layer was sutured in a simple continuous pattern using 4-0 polyglyconate suture, and the skin was closed in a simple interrupted pattern using 4-0 nylon. An additional 50 IU/kg of heparin was injected intravenously at 1 h after the first heparin injection.

During the first 3 postoperative days, enrofloxacin (5 mg/kg) and ketorolac (2 mg/kg) were injected subcutaneously to prevent infection and provide analgesia, respectively. The animals were evaluated daily for wound infection and hematoma formation for 1 week after surgery, and once per week thereafter. The control group (n = 8) was given 5 ml of normal saline and the ARB group (n = 8) was given 25 mg/kg of losartan potassium daily by oral administration for 6 weeks after surgery. This animal study was approved by the Institutional Animal Care and Use Committee of Yonsei University Health System (publication no. 2015-0023, 2015).

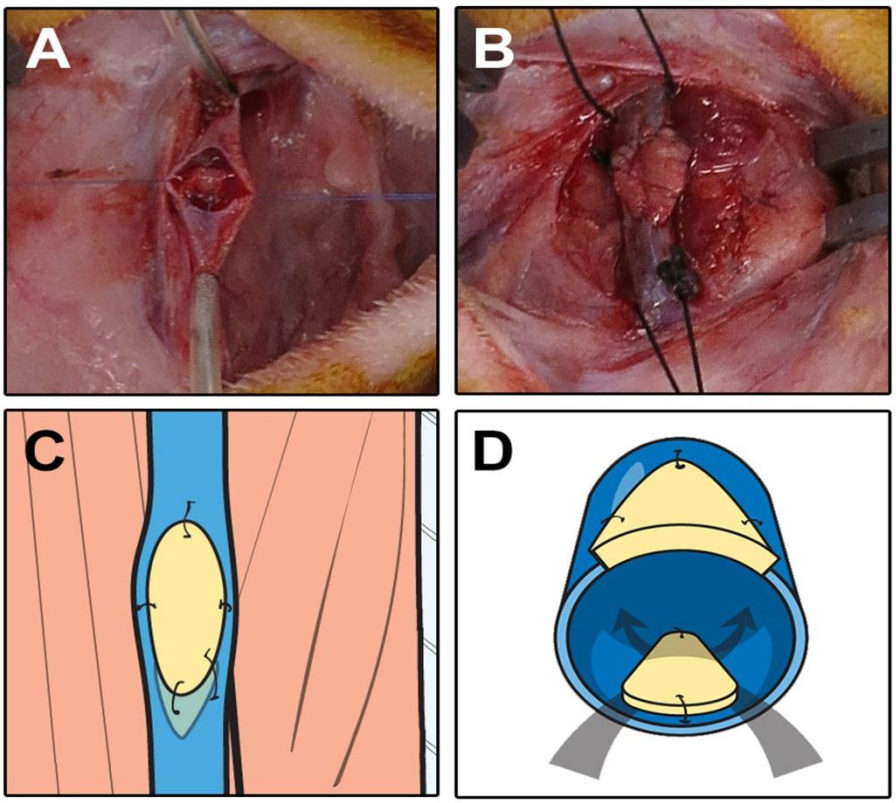

Figure 1. Gross appearance and illustration of the implantation model used in this study. (A) Image of intravenous implantation. (B) Image of patch angioplasty. (C) Illustration of intravenous implantation with patch angioplasty. (D) Cross-sectional view of intravenous implantation model.

3. Assessment of vascular patency

Vascular patency was confirmed just before euthanasia via angiography using computed tomography (CT; 16-channel multidetector; BrightSpeed Elite, GE Healthcare, Fairfield, CT, USA). Animals were anesthetized as described above, and then angiography was performed with a bolus intravenous injection of 2 ml/kg iohexol (Omnipaque ™, 300 mg I/ml).

4. Histology and immunohistochemistry

Animals were euthanized 6 weeks after surgery by bolus intravenous injection of potassium chloride under general anesthesia. Then, the BHV leaflet tissues were collected, including vessels and surrounding tissues. The BHV leaflet implant from the left jugular vein was used for quantitative calcium determination, and one-half of the BHV leaflet implant from the right jugular vein was used for each of two evaluations: histopathological examination and western blot analysis.

For histopathology, each sample was fixed in 10% neutralized buffered formalin (BBC Biochemical, WA, USA), further processed using standard methods, and embedded in paraffin. Then, 4 μm thick sections were stained with hematoxylin and eosin.

For immunohistochemistry, the sections were deparaffinized using standard protocols, and antigen retrieval was performed with proteinase K for CD31, smooth muscle α-actin (α-SMA). Then, sections were incubated for 10 min in 3%

hydrogen peroxide (Duksan Hydrogen peroxide 3059, Kyungkido, Korea) to inactivate endogenous peroxidase, and blocked by incubating for 1 h in 5% bovine serum albumin. Subsequently, the sections were labeled with the following primary mouse monoclonal antibodies (Abcam, Cambridge, UK): anti-CD31 (ab9498), anti-α-SMA (ab7817), anti-Ang II type 1 receptor (ab9391), and anti-bone morphogenic protein-2 (BMP-2, ab6285). The antibody-labeled sections were then incubated with Dako EnVision+ System-HRP labeled polymer anti-mouse kit solution (Dako4001, Denmark). DAP (K-3468, Dako, Denmark) staining was performed for 3 min at room temperature for tissue visualization. Sections were then counterstained with hematoxylin.

5. Quantitative calcium determination

Calcium measurements of the implants were performed using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Optima 8300, Perkin Elmer, Norwalk, CT, USA). For ICP-OES analysis, all surrounding connective tissues were cleanly removed from the implants, which were then frozen for 24 h at □80°C. Moisture was removed from the samples during a 24-h period by freeze-drying. Then, the implants were weighed and then suspended in 5 ml aqua regia in a 100 ml beaker. After adding 5 ml of hydrogen peroxide (H2O2), the beaker was slowly heated on a hot plate for 6 h at 70□80°C to dissolve the tissue, and then for a further 5 h at 140□150°C. An additional 5 ml of H2O2 was then added. Subsequently, each sample was diluted to 50 ml total with the addition of distilled water, and each diluted sample was used for the analysis of

calcium contents.

6. Western blot analysis

To investigate the expression of IL-6, BMP-2, and osteopontin, the BHV implant was separated from the vessel and stored at □80□C. Protein was extracted from the implants using 500 □l of PRO-PREP® protein extraction solution (Intron Biotechnology, Kyungki-Do, Korea) and tissue homogenization with sharp scissors. Samples were placed on ice for 30 min, and then were centrifuged at 14,000 rpm for 10 minutes at 4□C. The supernatant containing the protein lysates were collected.

Protein content was quantified with a protein assay kit (Bio-Rad, CA, USA). Then, 50 μg of total protein extract from each sample was mixed with Laemmli sample buffer and transferred to a Mini Protean® TGX™ precast gradient gel (Bio-Rad) with DOKDO-MARK™ (Intron Biotechnology, Kyungki-Do, Korea) as the standard prestained protein marker. Gel electrophoresis was performed with a Mini Protean® system (Bio-Rad) at 100 V for 130 minutes. After electrophoresis, proteins were blotted onto a methanol pre-activated PVDF membrane using a Mini Trans-Blot® Cell (Bio-Rad) at 230 mAh for 90 min. Blotted membranes were then blocked with 5% skimmed milk and incubated with anti-mouse monoclonal IL-6 antibody (1:1,000 dilution; Abcam, Cambridge, UK), anti-BMP-2 antibody (1:1,000 dilution; Abcam), and anti-osteopontin antibody (1:1,000 dilution; NOVUSBio, CO, USA) in PBS-T (0.5% tween20 in phosphate buffered saline) buffer containing 5% skimmed milk.

Separate blots were performed for each antibody. Anti-β-actin antibody was used as a loading control (1:2,000 dilution; Abcam). Membranes were incubated with the primary antibodies for 60 min at room temperature, and then washed three times with PBS-T buffer. Then, membranes were incubated with the secondary antibody for 60 min at room temperature (goat anti-mouse IgG-HRP, 1:2,000 dilution; Abcam). Membranes were then washed three times with PBS-T buffer, proteins were detected using an enhanced chemiluminescence (ECL) detection reagent for immunoblot analysis (Daeill Lab Service, Seoul, Korea). Subsequently, the ECL signals were quantitated using the pixel density analysis algorithm of ImageJ software (National Institute of Health, NY, USA). The relative band density was defined as follows: relative band density = (specific band density / β-actin band density) × 100.

7. Statistical analysis

All data were expressed as mean ± SD. Statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software, Inc., San Diego, CA, USA). The unpaired t-test was used to compare two groups.All statistical tests were two-sided, and significance was defined as $p<0.05$.

III. RESULTS

1. Calcium level quantification

There were no deaths during surgery. Side effects such as wound infection, hematoma formation, and ocular swelling due to jugular vein occlusion were not observed. All animals were euthanized on the planned date. Vascular patency was maintained without narrowing, which was confirmed in CT images at 6 weeks after surgery (Figure 2). Calcified lesions were not observed around the vessel or at the implants in both control and treatment groups. The Ca2+ level in the group treated with the ARB losartan (2.28 ± 0.65 mg/g) was significantly lower than that in the control group (3.68 ± 1.00 mg/g) ($p = 0.0092$, Figure 3). In the case of the tissue before implantation, the Ca2+ was not detected.

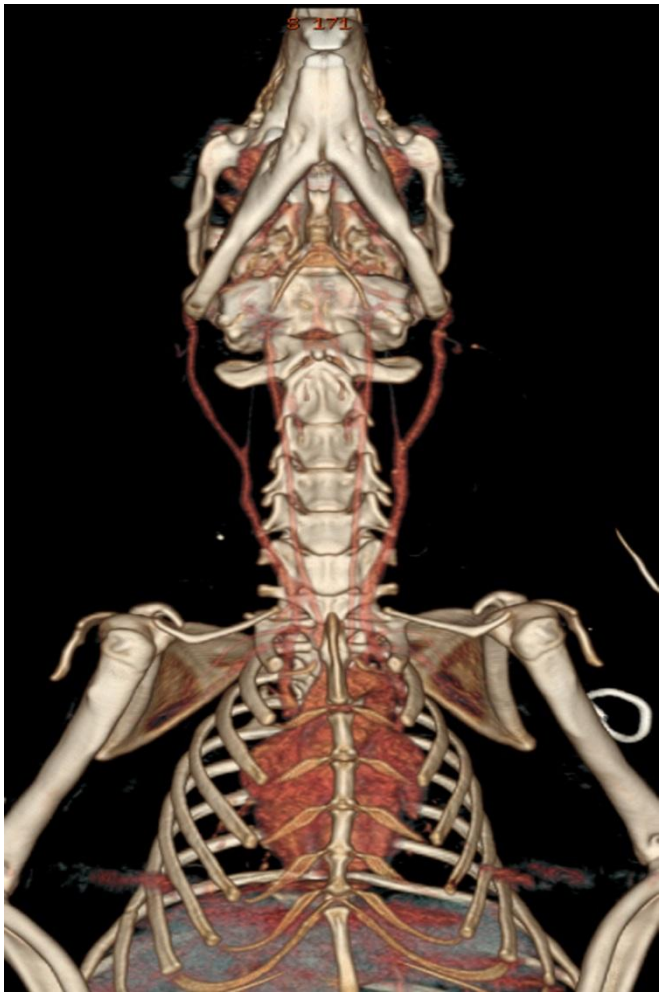

Figure 2. Three-dimensional reconstruction of vascular structures using computed tomography. The patency of both external jugular veins was maintained without narrowing.

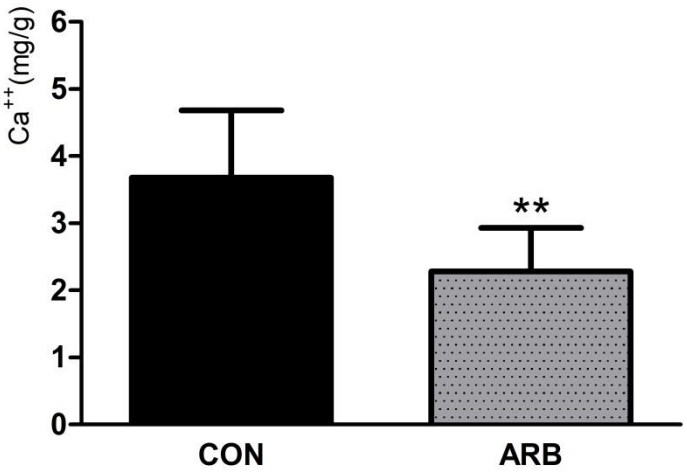

Figure 3. Quantitative analysis of calcium contents in bovine pericardial implants. The calcium level of the losartan-treated group was significantly lower than that of the in control group. CON: control group; ARB: losartan-treated group; ** $p < 0.01$.

2. Histological results

Histological examination revealed that BHV leaflet appeared stacked eosinophilic collagen fibers with sparse fibroblast (Figure 4). Numerous cellular infiltrates including lymphoid cells, eosinophils, histiocytes and (myo)fibroblasts were observed around the implants. Histiocytes and fibroblasts were present at implant borders and some of them infiltrated between the collagen fibers in the periphery. The histologic features were similar in both control and ARB-treated groups.

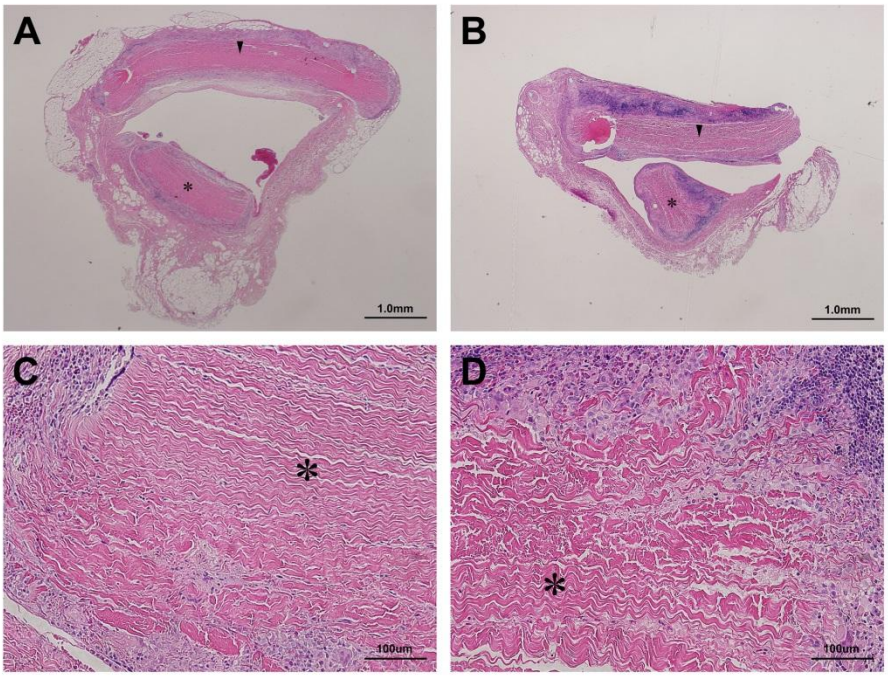

Figure 4. Microscopy of bovine pericardial implants in control group (A and C) and losartan-treated group (B and D) visualized with hematoxylin and eosin stain. Venous patch (arrow head), intravenous implant (asterisk), and vessel lumen can be seen in the lower-magnification images. Numerous cellular infiltrates are observed around and within the implant. A and B, × 40 magnification; C and D, × 200 magnification.

3. Immunohistochemical staining

Similar immunohistochemical staining patterns were also observed in the control and ARB-treated groups for α-SMA, Ang II type 1 receptor, and CD 31 (Figure 5). Many of the infiltrated cells showed a positive reaction for Ang II type 1 receptor antibody and there are also a lot of α-SMA positive cells. Most of the infiltrated cells showing a positive reaction for Ang II type 1 receptor were macrophages. In addition, immunohistochemical staining patterns for BMP-2 significantly differed between the control and ARB-treated groups (Figure 6). Most infiltrated cells showed positive reaction to the BMP-2 antibody in control group. However, only a few BMP-2 positive cells were observed in the ARB-treated group.

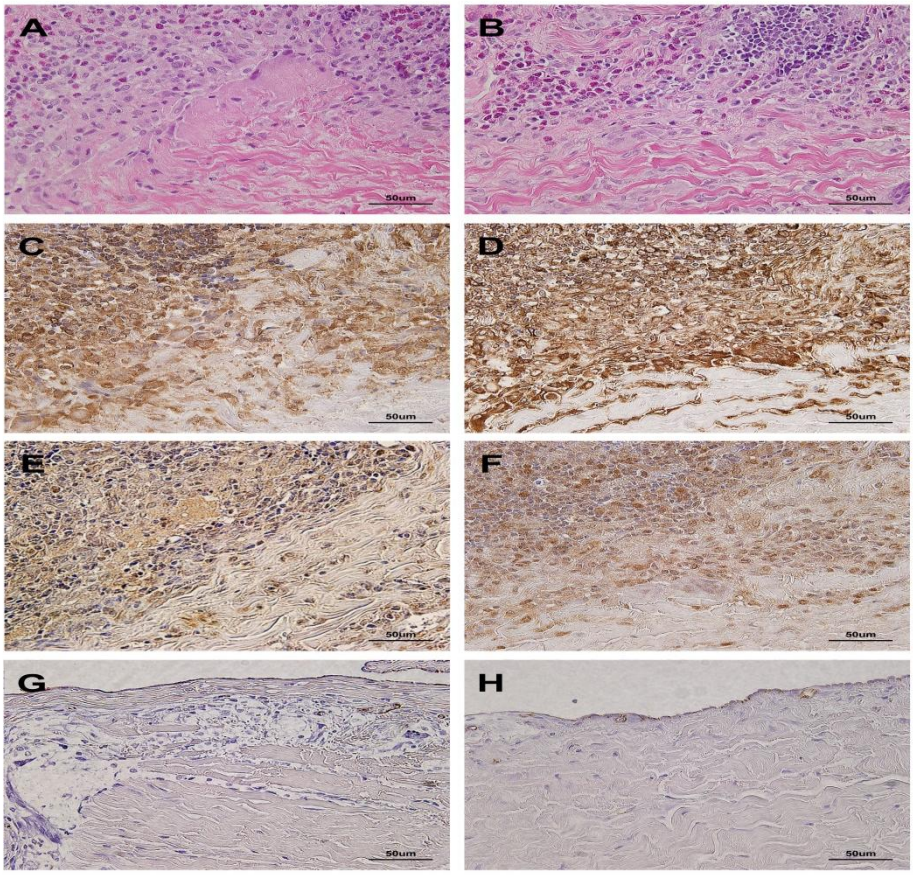

Figure 5. Bovine pericardial implants in the control group (A, C, E, and G) and the losartan-treated group (B, D, F, and H) were visualized with hematoxylin and eosin staining and immunohistochemistry. There are a lot of α-SMA positive cells (C and D) and many of the infiltrated cells express the Ang II type I receptor (E and F). CD31-positive cells (endothelial cells) line the implants near the vessel lumen (G and H).

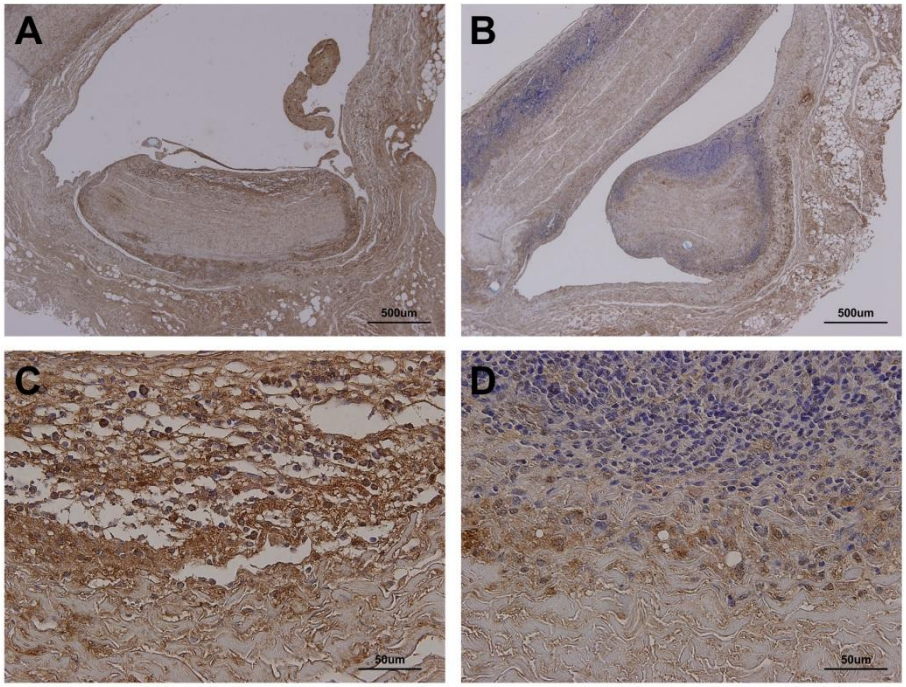

Figure 6. Immunohistochemistry of bone morphogenic protein-2 (BMP-2) in the control group (A and C) and the losartan-treated group (B and D). A lot of BMP-2 positive cells (cytoplasmic brown staining) can be seen in the control group (A and C). By contrast, BMP-2 positive cells in the losartan-treated group were significantly lower than those in the control group (B and D).

4. Western blot analysis

Western blot analysis showed that ARB significantly suppressed IL-6 ($p < 0.05$), osteopontin ($p < 0.001$), and BMP-2 ($p < 0.05$) expression. The relative band densities of the control and ARB-treated groups were as follows: 118.13 ± 64.53% and 47.04 ± 24.86%, respectively, for IL-6; 122.61 ± 10.50% and 21.58 ± 14.01%, respectively, for osteopontin; and 159.69 ± 59.80% and 75.57 ± 22.38%, respectively, for BMP-2 (Figure 7).

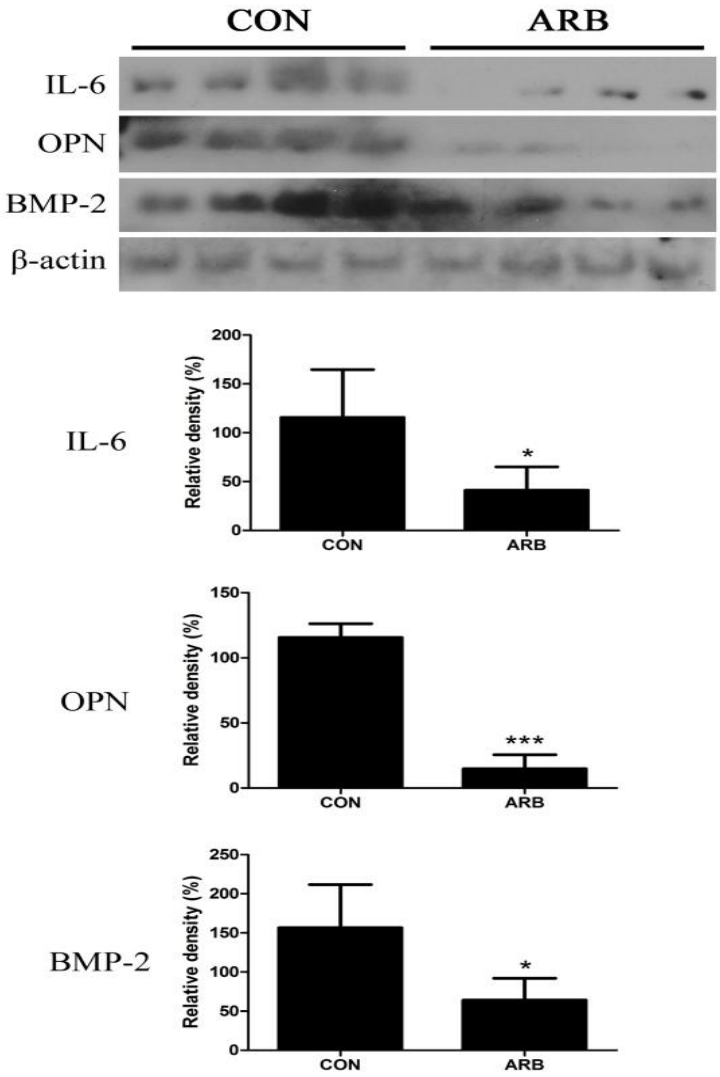

Figure 7. Western blot analysis of bovine pericardial implants. Interleukin-6 (Il-6), osteopontin (OPN), and BMP-2 expression is significantly lower in the losartan-treated group than in the control group. CON, control group; ARB, losartan-treated group; * $p < 0.05$; *** $p < 0.001$.

IV. DISCUSSION

Human heart valves can be successfully replaced with bioprosthetic valves; however, eventual degeneration of the valve leaflet due to calcification is a concern. Several studies have reported a relationship between angiotensin II and degenerative aortic stenosis with calcification.[9-11, 13-15]. To our knowledge, there are no reports about the relationship between angiotensin II and bovine pericardial xenograft calcification. The present study revealed that the ARB losartan significantly attenuated post-implant degenerative calcification of bovine pericardial bioprosthesis. The study also determined that the mechanism of bovine pericardial bioprosthesis calcification was similar to that of native valve calcification. Degenerative calcification of bioprosthesis xenograft is not a phenomenon composed of a single mechanism, but involves various mechanisms such as mechanical factor, chemical cross-linking and immune responses.[22] It is thought that mechanical damage and chemical cross-linking affects cells and cell fragments in bioprosthetic tissue. Then, Ca2+ in blood and interstitial fluid moves into the affected cells and interacts with cell phosphorus, which is present in cell membranes, to initiate calcium phosphate crystal nucleation. Finally, calcium phosphate crystal growth expands and eventually blocks valve movement.[1] Many strategies have been tested to prevent the problems of calcification, including removal of all cellular components of bovine pericardium, modification of glutaraldehyde crosslinking, and

immunological modification; however, none of these methods appear to be superior, and the studies are still ongoing.

Many studies have investigated calcification of the aortic valve and arteries to elucidate the relationship between Ang II and valve calcification,[9-13] and to evaluate a preventive role for ACE inhibitor or ARB on calcification.[13, 19] Ang II has a number of potential lesion-promoting effects, such as those mediated by the Ang II type 1 receptor. These effects include stimulating inflammation and macrophage cholesterol accumulation, impairing fibrinolysis, and increasing oxidative stress.[14] Also, Ang II-mediated upregulation of BMP-2 expression in valvular fibroblasts or myofibroblasts can lead to osteoblastic differentiation of fibroblasts and calcium deposition. [15] ARB can block this fibroblast differentiation by inhibiting the Ang II receptor. In addition, there are some evidences that local inflammatory response alone with increasing the IL-6 is associated with increasing the BMP-2 expression and the osteopontin level in valvular degenerative calcification process.[23] Osteopontin is also known for involving the calcification of bioprosthesis. [24] In addition, Côté et al. reported that losartan can reduce the IL-6 expression on calcific aortic valve.[19] Based on these results of researches, we focused on the investigation of IL-6, osteopontin, and BMP-2 expressions.

On the other hands, some studies have suggested that residual antigens of the bioprosthesis could cause humoral and cellular immune responses, leading to mineralization process.[22] Thus, we hypothesized that ARB-mediated reduction

of inflammation also could reduce bioprosthetic valve calcification. In this study, most of the infiltrated cells showing a positive reaction for AngII type 1 receptor were macrophages and the cells positive for α-SMA antibody, and BMP-2 expression was reduced in these cells of the ARB-treated group compared with control group. These results suggest that macrophages and (myo)fibroblast cells infiltrating into the decellularized bioprosthetic valve have AngII type 1 receptors, and ARB treatment might reduce the BMP-2 expression in the macrophages. Western blot analysis revealed that the ARB losartan significantly suppressed IL-6, osteopontin, and BMP-2 expression. Reduced IL-6 level in the ARB-treated group is probably related to reduced inflammation process of macrophages mediated by Ang II. Thus, ARB can reduce calcification by reducing inflammation process of macrophages and blocking osteoblastic differentiation of (myo)fibroblasts. These results are compatible with the significant decrease of calcium content in the ARB-treated group compared with control group.

The limitations of this study are the small number of experimental animals and the uncertainty regarding the application of degenerative calcification mechanisms of bovine pericardial leaflet in a rabbit intravascular implant model to the calcification mechanisms of a bioprosthetic valve. Bioprosthetic valve leaflets implanted in the aortic or pulmonic position are effectually exposed to higher pressure and larger flow volume than those of intravenous implanted leaflets. However, Meuris et al. implanted glutaraldehyde-fixed porcine cusps in both jugular vein and carotid artery and they concluded that the venous implants

significantly increased the calcification of cusp in the sheep.[25] In addition, we compared five implantation methods in a rabbit model, and found that the intravenous implant model most closely reflected bioprosthetic valve calcification and the calcium measurement result was higher in intravenous implantation than in arterial patch implantation. [20] We believe that this model is appropriate to evaluate ARB effects on bovine pericardium calcification because it is easier than implantation of a bioprosthetic valve in a large animal. This model is also thought to be more useful to screening the anticalcification effects of various drugs which deliver via blood stream because its characteristics of sufficient blood contact compared with rodent subcutaneous implantation model or rabbit intramuscular implantation model.

V. CONCLUSION

This study is the first to report that losartan significantly attenuated post-implant degenerative calcification of a bovine pericardial bioprosthesis in a rabbit intravascular implant model. Further observations are required to assess ARB effects on bioprosthetic heart valve tissue in an orthotopic implantation using a large animal model.

REFERENCES

1. Schoen FJ, Levy RJ. Calcification of tissue heart valve substitutes: progress toward understanding and prevention. Ann Thorac Surg 2005;79:1072– 80.

2. McClure RS, Narayanasamy N,Wiegerinck E, Lipsitz S, Maloney A, Byrne JG, et al. Late outcomes for aortic valve replacement with the Carpentier–Edwards pericardial bioprosthesis: up to 17-year follow-up in 1,000 patients. Ann Thorac Surg 2010;89:1410-6.

3. Lee C, Park CS, Lee CH, Kwak JG, Kim SJ, Shim WS, et al. Durability of bioprosthetic valves in the pulmonary position: long-term follow-up of 181 implants in patients with congenital heart disease. J Thorac Cardiovasc Surg 2011;142:351-8.

4. Kwak JG, Lee JR, Kim WH, Kim YJ. Mid-term results of the Hancock II valve and Carpentier–Edward Perimount valve in the pulmonary position in congenital heart disease. Heart Lung Circ 2010;19:243-6.

5. Levy RJ, Schoen FJ, Lund SA, Smith MS. Prevention of leaflet calcification of bioprosthetic heart valves with diphosphonate injection therapy. Experimental studies of optimal dosages and therapeutic durations. J Thorac Cardiovasc Surg 1987;94:551–7.

6. Levy RJ, Wolfrum J, Schoen FJ, Hawley MA, Lund SA, Langer R. Inhibition of calcification of bioprosthetic heart valves by local controlled release diphosphonate. Science 1985;71:349 –56.

7. Levy RJ, Vyavahare N, Ogle M, Ashworth P, Bianco R, Schoen FJ. Inhibition of cusp and aortic wall calcification in ethanol- and aluminum-treated

bioprosthetic heart valves in sheep: background, mechanisms, and synergism. J Heart Valve Dis 2003;12:209 –16.

8. Kim KC, Kim SH, Kim YJ. Detoxification of Glutaraldehyde Treated Porcine Pericardium Using L-arginine & NABH(4). Korean J Thorac Cardiovasc Surg 2011;44:99-107.

9. O'Brien KD, Probstfield JL, Caulfield MT, Nasir K, Takasu J, Shavelle DM, et al. Angiotensin-converting enzyme inhibitors and change in aortic valve calcium. Arch Intern Med 2005;165:858-62.

10. Arishiro K, Hoshiga M, Negoro N, Jin D, Takai S, Miyazaki M, et al. Angiotensin receptor-1 blocker inhibits atherosclerotic changes and endothelial disruption of the aortic valve in hypercholesterolemic rabbits. J Am Coll Cardiol 2007;49:1482-9.

11. Ngo DT, Stafford I, Sverdlov AL, Qi W, Wuttke RD, Zhang Y, et al. Ramipril retards development of aortic valve stenosis in a rabbit model: mechanistic considerations. Br J Pharmacol 2011;162:722-32.

12. Armstrong ZB, Boughner DR, Drangova M, Rogers KA. Angiotensin II type 1 receptor blocker inhibits arterial calcification in a pre-clinical model. Cardiovasc Res 2011;90:165-70.

13. Côté N, Couture C, Pibarot P, Després JP, Mathieu P. Angiotensin receptor blockers are associated with a lower remodelling score of stenotic aortic valves. Eur J Clin Invest 2011;41:1172-9.

14. O'Brien KD, Shavelle DM, Caulfield MT, McDonald TO, Olin-Lewis K, Otto CM, et al. Association of angiotensin-converting enzyme with low-density lipoprotein in aortic valvular lesions and in human plasma. Circulation

2002;106:2224–30.

15. O'Brien KD. Pathogenesis of calcific aortic valve disease: a disease process comes of age (and a good deal more). Arterioscler Thromb Vasc Biol 2006;26:1721-8.

16. Rajamannan NM, Otto CM. Targeted therapy to prevent progression of calcific aortic stenosis. Circulation 2004;110:1180-2.

17. Walia DS, Sharma M, Raveendran VV, Zhou J, Sharma R, Stechschulte DJ, et al. Human mast cells (HMC-1 5C6) enhance interleukin-6 production by quiescent and lipopolysaccharide-stimulated human coronary artery endothelial cells. Mediators Inflamm 2012;2012:274347.

18. Klinghammer L, Urschel K, Cicha I, Lewczuk P, Raaz-Schrauder D, Achenbach S, et al. Impact of telmisartan on the inflammatory state in patients with coronary atherosclerosis--influence on IP-10, TNF-α and MCP-1. Cytokine 2013;62:290-6.

19. Côté N, Mahmut A, Fournier D, Boulanger MC, Couture C, Després JP, et al. Angiotensin receptor blockers are associated with reduced fibrosis and interleukin-6 expression in calcific aortic valve disease. Pathobiology 2014;81:15-24.

20. Kim D, Park HK, Park YH, Lee SH, Joo HC, Lee S, et al. Degenerative Calcification of Pericardial Bioprostheses: Comparison of Five Implantation Methods in a Rabbit Model. The Journal of Heart Valve Disease 2015;24:621-8.

21. Xu YM, Sharma D, Li GP, Zhao YN. Effect of Angiotensin II Type 1 Receptor Antagonist, Losartan on Inflammatory Factor in Atherosclerotic Rabbits. Res Cardiovasc Med 2013;2:127-32.

22. Pibarot P and Dumesnil JG. Prosthetic heart valves: selection of the optimal prosthesis and long-term management. Circulation 2009;119:1034-48.

23. Hjortnaes J, Butcher J, Figueiredo JL, Riccio M, Kohler RH, Kozloff KM, et al. Arterial and aortic valve calcification inversely correlates with osteoporotic bone remodeling: a role for inflammation. Eur Heart J 2010;31:1975-84.

24. Srivatsa SS, Harrity PJ, Maercklein PB, Kleppe L, Veinot J, Edwards WD, et al. Increased cellular expression of matrix proteins that regulate mineralization is associated with calcification of native human and porcine xenograft bioprosthetic heart valves. J Clin Invest 1997;99:996-1009.

25. Meuris B, Ozaki S, Herijgers P, Verbeken E, Flameng W. Bioprosthetic tissue calcification: influence of blood contact and arterial pressure. an experimental study in rats and sheep. J Heart Valve Dis 2003;12:392-9.

ABSTRACT (IN KOREAN)

Angiotensin II receptor blocker인 Losartan이 토끼혈관내에 심은 조직판막의 석회화를 경감시킨다

<지도교수 박 영 환>

연세대학교 대학원 의학과

신 홍 주

심장조직판막의 퇴행적 석회화는 젊은 성인이나 소아에서 특히 문제점이다. 우리는 Angiotensin II receptor blocker인 Losartan이 토끼혈관내에 심은 조직판막의 석회화에 미치는 영향을 조사하였다. 총 16마리의 생후 20주의 2.98-3.34kg의 New Zealand White rabbits의 양측 경정맥에 3mm 크기의 조직판막을 심었다. 실험군 (8마리)에게는 25mg/kg 용량의 losartan을, 대조군 (8마리) 에게는 5ml의 생리 식염수를 6주동안 매일 먹였다. 6주후에 토끼를 희생하여 얻은 조직판막에서 칼슘량, 조직학적, 면역화학적, Western blot 검사를 시행하였다. 실험기간동안 감염이나 혈종 등 합병증은 없었으며, 죽은 토끼도 없었다. 실험군에서 칼슘량이 통계적으로 유의하게 적게 검출되었다 (p = 0.0281). 면역화학적 검사에서 bone morphogenic

protein 2 (BMP-2) 양성 반응이 실험군이 작게 나타났으며, Western blot 검사에서 losartan이 interlukin-6, osteopontin, BMP-2의 발현을 억제함을 확인할 수 있었다. 본 연구결과는 losartan이 토끼혈관내에 심은 조직판막의 석회화를 유의하게 경감시킴을 밝혔다. Angiotensin II receptor blocker의 심장조직판막의 석회화에 미치는 영향을 확인하기 위해서는 중대형동물에게 조직판막 치환술을 시행하여 조사해 볼 필요가 있다.

핵심되는 말: 심장조직판막, 퇴행적 석회화, 토끼