Background: Gallic acid (3,4,5-trihydroxybenzoic acid) is a natural polyphenol and strong natural antioxidant found abundantly in red wine and green tea. The aim of this study was to examine the anti-inflammatory effect of a novel gallic acid-eluting stent in a porcine coronary restenosis model.

Methods: Fifteen pigs were randomized into three groups; in which a total of 30 coronary arteries (10 in each group) were implanted with gallic acid-eluting stents (GESs, n = 10), gallic acid and sirolimus-eluting stents (GSESs, n = 10), or sirolimus-eluting stents (SESs, n = 10). Histopathologic analysis was performed 28 days after stenting.

Results: There were no significant differences in injury score and fibrin score among the groups, however there were significant differences in the internal elastic lamina (4.0 ± 0.83 mm² in GES vs. 3.0 ± 0.53 mm² in GSES vs. 4.6 ± 1.43 mm² in SES, p < 0.0001), lumen area (2.3 ± 0.49 mm² in GES vs. 1.9 ± 0.67 mm² in GSES vs. 2.9 ± 0.56 mm² in SES, p < 0.0001), neointimal area (1.7 ± 0.63 mm² in GES vs. 1.1 ± 0.28 mm² in GSES vs. 1.7 ± 1.17 mm² in SES, p < 0.05), and percent area of stenosis (42.4% ± 9.22% in GES vs. 38.2% ± 12.77% in GSES vs. 33.9% ± 15.64% in SES, p < 0.05). The inflammation score was significantly lower in the GES and GSES groups compared to that in the SES group [1.0 (range: 1.0 to 2.0) in GES vs. 1.0 (range: 1.0 to 1.0) in GSES vs. 1.5 (range: 1.0 to 3.0) in SES, p < 0.05].

Conclusions: The GES group had a greater percent area of stenosis than the SES group. Although gallic acid in the GES and GSES groups did not show a synergistic effect in suppressing neointimal hyperplasia, it resulted in greater inhibition of the inflammatory reaction in the porcine coronary restenosis model than in the SES group.

Key Words: Inflammation • Percutaneous coronary intervention • Restenosis • Stent

INTRODUCTION

Coronary artery atherosclerosis and occlusion are among the leading causes of morbidity and mortality in industrialized countries. Common treatment of coronary occlusion includes balloon angioplasty (BA) and bare-metal stent (BMS) and/or drug-eluting stent (DES) implantation. Recently, biodegradable scaffolds and polymer-free DESs have been developed and used for treatment.1,3

Sirolimus (CYPHER®, Cordis Corporation, Johnson & Johnson, Warren, NJ, USA) and paclitaxel (Taxus®, Boston Scientific, Boston, MA, USA) eluting stents were introduced as first-generation intracoronary DESs. Sirolimus, also known as rapamycin, was first isolated from streptomyces hygroscopius.4 Rapamycin is commonly
used to coat coronary stents, and it is an antibiotic with potent antiproliferative, immunosuppressive, and anti-migratory properties.

Taxol (generic name: paclitaxel) is isolated from the evergreen Pacific yew (Taxus brevifolia). Paclitaxel has anti-vascular smooth muscle cell (VSMC) proliferation and anti-cancer effects. Thus, the first-generation of DESs with paclitaxel were used in patients with acute myocardial infarction to prevent in-stent restenosis. This was the first plant-based substance used in a coronary stent.

Although the advent of DESs has reduced restenosis rates by 50-90% compared with BA and BMSs, DESs are associated with several limitations including late stent thrombosis (LST) and chronic inflammation at the stented lesion. Sirolimus-eluting stents (SESs) have been reported to have superior clinical outcomes to paclitaxel-eluting stent in comparative trials. Gallic acid (3,4,5-trihydroxybenzoic acid) is a phenolic acid and a phytochemical. It has been shown to have anti-inflammatory, antioxidative, antimicrobial, and anticancer effects. Sirolimus and gallic acid have different mechanisms of inhibiting inflammation and neointimal hyperplasia. Gallic acid induces apoptosis and suppresses VSMCs by producing the hydroxyl radical. Sirolimus has also been shown to suppress the proliferation of VSMCs by inhibiting cell cycle-dependent kinases and delaying the phosphorylation of retinoblastoma protein, suggesting that a synergistic effect may be achieved by combining sirolimus and gallic acid. Therefore, in this study, we investigated the inhibitory effect of gallic acid-eluting stents (GESs) and gallic acid and sirolimus-eluting stents (GSESs) on vascular inflammation and smooth muscle cell growth in a porcine coronary restenosis model.

METHODS

Materials

Poly-DL-lactide (PLLA; 0.80-1.2 dL/g of inherent viscosity in chloroform at 0.1 w/v% at 25 °C) and poly (D-L-lactide-co-glycolide) at a ratio of 50:50 (0.45-0.60 dL/g of inherent viscosity in chloroform at 0.1 w/v% at 25 °C) was purchased from EVONIK (UK). Sirolimus was purchased from LC Laboratories (USA). Phosphate-buffered saline (PBS) and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and solvents were of analytical grade and used without further purification.

Preparation of gallic acid-, gallic acid and sirolimus-, and sirolimus-eluting stents (Figure 1)

The BMS (Chonnam National University Hospital stent) used in the study was made by laser-cut processing of a cobalt-chromium alloy tube (L605 Co-Cr alloy) followed by electropolishing to a strut thickness of < 70 μm. An ultrasonic spray method was used to apply coatings to the prepared BMS (3 × 16 mm). The required amount of poly (L-lactide) (PLA) and gallic acid was dissolved in 5 mL of tetrahydrofuran (THF), and the drug solution was then dissolved in the polymer solution. The stents to be coated were cleaned with ethanol and distilled water and then vacuum-dried for 24 h. The sprayed liquid consisted of the polymer/drug solution dissolved in THF and diluted to 2% by weight. Coating application required a flow rate of 50 μL/min. The stents were placed on a mandrel attached to a rotating shaft, coated, and vacuum-dried for 24 h. The surface morphologies of the GESs, GSESs, and SESs were then examined.

Coated stent evaluation

We used a scratch method under a scanning electron microscopy (SEM; SNE-1500M, SEC Co., Ltd., Korea) and reflection spectrometry (RS; F40, Filmetrics, Inc., USA) using a wavelength range of 400-850 nm to measure the thickness of the coated layer on each stent. The optical index of refraction was assumed to be n = 1.50 of PLA. To determine the total amount of gallic acid on a BMS, the uncoated area of the stent was calculated by SEM images. Then, the total amount of gallic acid in the coated area was calculated based on the thickness of the coated layer and the surface area of the stent. This method was also used to determine the total amount of sirolimus in the coated area. Figure 1. Schematic illustration of the GES, GSES, and SES. GES, gallic acid-eluting stent; GSES, gallic acid- and sirolimus-eluting stent; SES, sirolimus-eluting stent.
the coated stent was sonicated in 5 mL of acetonitrile (ACN) for 1 h to dissolve the coated layer with the drug and then analyzed using an ultraviolet-visible spectrophotometer (UV; UV-1800, Shimadzu, Japan) at 241.5 nm. In vitro drug release was measured using a simple shaking method with UV.22

All stents were expanded to a 3.0-mm diameter, and then immersed in 5 mL of phosphate buffered saline (PBS) in colored vials and subjected to 100 rpm shaking at 37 °C. The stent was taken out at each designated time point, and the PBS was replaced with fresh solution at the specified times. The drugs remaining on the stents were dissolved in ACN and measured with a UV-visible spectrophotometer at 241.5 nm.

Animal preparation and stent implantation

The study animals were Yorkshire x Landrace F1 crossbred, castrated male swine with an average age of 7-9 weeks. The animal experiments for coronary stenting were conducted as described previously.23 Pigs were selected randomly in this study and the coronary artery size was highly variable. Therefore, the balloon pressure was adjusted according to the vessel size to accommodate the stent diameter. The stent was deployed by inflating the balloon (3 × 20 mm) and the resulting stent-to-coronary artery ratio was 1.3:1. The diameter of the implanted coronary stent (stent-to-artery ratio) was adjusted with reference to the 7-F guiding catheter diameter (2.31 mm). The stented pigs underwent follow-up angiography after 4 weeks. The pigs were anesthetized on the day of follow-up with zolazepam and tiletamine (2.5 mg/kg; Zoletil50®, Virbac, Carros, France), xylazine (3 mg/kg; Rompun®, Bayer AG, Leverkusen, Germany), and azaperone (6 mg/kg; Stresnil®, Janssen-Cilag, Neuss, Germany). The pigs were sacrificed with 20 mL of potassium chloride by intracoronary injection under deep anesthesia after follow-up angiography.

Study groups

The pigs were randomly divided into three groups: GES (3.0 × 16 mm, n = 10), GSES (3.0 × 16 mm, n = 10), and SES (3.0 × 16 mm, n = 10) groups. A total of 15 pigs (30 coronary arteries) were used in this study. A GES, GSES, or SES was implanted in the left anterior descending and left circumflex artery of each pig in a randomized manner.

Histopathological and micro-computed tomography analysis

Histopathological evaluation of each artery was performed by an experienced cardiovascular pathologist. The specimens were embedded, and sections of ~3-5-μm thickness were obtained at ~1-mm intervals and stained with hematoxylin and eosin (H&E) and Carstairs’ stain for histological analysis. The histopathological sections were measured using a calibrated microscope, digital video imaging system, and microcomputer program (Visus 2000 Visual Image Analysis System, IMT Tech, CA, USA). Borders were manually traced for lumen area, and the area was circumscribed by the internal elastic lamina and the innermost border of the external elastic lamina (i.e., external elastic lamina area). Morphometric analysis was used to calculate the neointimal area of a given vessel as the measured internal elastic lamina area minus the lumen area. Measurements were made on five cross sections from proximal and distal ends, and three midpoints of each stented segment. Histopathological stenosis was calculated as 100 x [1 – (lesion lumen area/lesion internal elastic lamina area)].24 The harvested stent specimens were stored in formaldehyde solution. A 1.5-mL Eppendorf tube was filled with clay, and the clay was formed into a V shape to hold the stent during contrast agent staining. The stents were taken from the solution and placed vertically in the V-shaped opening in the clay. Each stent had to be fixed in the clay so that it would not move inside the tube. The contrast agent used was omnihexol. One milliliter of the contrast agent was then placed in a 5-mL syringe and injected through the opening at the center of the stent. The stent was incubated with the contrast agent overnight and subjected to micro-computed tomography (CT) imaging.25 All results were interpreted by two independent pathologists in a blinded fashion.

Classification of in-stent restenosis using angiographic patterns

Coronary angiograms were reviewed by independent pathologists who classified the lesions according to the following criteria (Table 1).26

Evaluation of arterial injury score

Arterial injury at each stent strut site was determined according to the anatomic structures penetrated by each stent strut. A numeric value was assigned as previously
described by Schwartz et al.: 0 = no injury; 1 = break in the internal elastic membrane; 2 = perforation of the media; and 3 = perforation of the external elastic membrane to the adventitia.24 The average injury score for each segment was calculated by dividing the sum of the injury scores by the total number of stent struts at the examined section (Table 2).27

**Evaluation of inflammation scores and fibrin scores**

The inflammation score for each individual stent strut was graded as follows: 0 = no inflammatory cells surrounding the stent strut; 1 = light, noncircumferential lymphohistiocytic infiltration surrounding the strut; 2 = localized, noncircumferential, moderate-to-dense cellular aggregates surrounding the stent strut; and 3 = circumferential, dense lymphohistiocytic cell infiltration of the stent strut. The inflammation score for each cross section was calculated by dividing the sum of the individual inflammation scores by the total number of stent struts at the examined section (Table 2).28 Ordinal data for fibrin were collected for each stent section using a scale of 0-3 as previously reported (Table 2).29

**Ethical statement**

This animal study was approved by the Ethics Committee of Chonnam National University Medical School and Chonnam National University Hospital (CNU IACUC-H-2016-03), and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

**Statistical analysis**

Statistical analysis was performed with commercially
available software (SPSS Version 15, Chicago, IL, USA). Data were presented as mean ± SD. The unpaired Student’s t test was used to compare each stent group, and analysis of variance (ANOVA) was used for comparisons of the three stent groups. Ordinal measurements such as injury, fibrin, and inflammation scores were analyzed using the Kruskal-Wallis test. The Mann-Whitney test was used to compare ordinary values in each stent group. Non-parametric results were presented as median and interquartile range. p values < 0.05 were considered to be statistically significant.

RESULTS

Surface coating and drug-releasing evaluation of the GES, GSES, and SES

Roughness and defects of the polymer-coated surface on the stent have been reported to potentially affect stent thrombosis and drug release.30,31 Our SEM findings (Figure 2) showed that the coated surface was uniform and smooth, and neither bridging nor webbing was observed in any of the stents. The coating thickness using RS was 5-8 µm. The in vitro elution of gallic acid and sirolimus from the coated stents is shown in Figure 3. Gallic acid and sirolimus were released for more than 25 days.

Stent implantation in pigs

A total of 30 stents (10 GESs, 10 GSESs, and 10 SESs) were placed in the left anterior descending and left circumflex arteries of 15 pigs. The overall mortality rate was 0% in this study. There were no significant differences in stent-to-artery ratio among the three stent groups.

Follow-up coronary angiographic findings

All stented coronary arteries showed in-stent restenosis pattern II on follow-up coronary angiographic analysis.

Histopathological findings in the three groups (Table 3)

There were no significant differences in injury score [1.0 (range: 1.0 to 1.0) in GES vs. 1.0 (range: 1.0 to 1.0) in GSES vs. 2.0 (range: 1.0 to 2.0) in SES, p = NS] or fibrin score [1.0 (range: 1.0 to 2.0) in GES vs. 2.0 (range: 1.0 to 2.0) in GSES vs. 2.0 (range: 0.0 to 3.0) in SES, p = NS] among the three groups. There were significant differences in internal elastic lamina (4.0 ± 0.83 mm² in GES vs. 3.0 ± 0.53 mm² in GSES vs. 4.6 ± 1.43 mm² in SES, p < 0.0001), lumen area (2.3 ± 0.49 mm² in GES vs. 1.9 ± 0.67 mm² in GSES vs. 2.9 ± 0.56 mm² in SES, p < 0.0001), neointimal area (1.7 ± 0.63 mm² in GES vs. 1.1 ± 0.28 mm² in GSES vs. 1.7 ± 1.17 mm² in SES, p < 0.05), and percent area of stenosis (42.4 ± 9.22% in GES vs. 38.2 ± 12.77% in GSES vs. 33.9 ± 15.64% in SES, p < 0.05) among the three groups. GESs achieved a greater percent area of stenosis than SESs.

The inflammation score [1.0 (range: 1.0 to 2.0) in GES vs. 1.0 (range: 1.0 to 1.0) in GSES vs. 1.5 (range: 1.0 to 3.0) in SES, p < 0.05] was significantly lower in the
GES and GSES groups compared to that in the SES group (Figure 4 and 5). Injury, fibrin, and inflammation scores were expressed as median (interquartile range).

### Table 3. Coronary artery morphometric measurements in 30 stented vessels

<table>
<thead>
<tr>
<th></th>
<th>GES (n = 10, A)</th>
<th>GSES (n = 10, B)</th>
<th>SES (n = 10, C)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury score</td>
<td>1.0 (1.0-1.0)</td>
<td>1.0 (1.0-1.0)</td>
<td>1.0 (1.0-2.0)</td>
<td>NS</td>
</tr>
<tr>
<td>IEL (mm²)</td>
<td>4.0 ± 0.83</td>
<td>3.0 ± 0.53</td>
<td>4.6 ± 1.43</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lumen area (mm²)</td>
<td>2.3 ± 0.49</td>
<td>1.9 ± 0.67</td>
<td>2.9 ± 0.56</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Neointima area (mm²)</td>
<td>1.7 ± 0.63</td>
<td>1.1 ± 0.28</td>
<td>1.7 ± 1.17</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>% area stenosis</td>
<td>42.4 ± 9.22</td>
<td>38.2 ± 12.77</td>
<td>33.9 ± 15.64</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Fibrin score</td>
<td>1.0 (1.0-2.0)</td>
<td>2.0 (1.0-2.0)</td>
<td>2.0 (0.0-3.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Inflammation score</td>
<td>1.0 (1.0-2.0)</td>
<td>1.0 (1.0-1.0)</td>
<td>1.5 (1.0-3.0)</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Injury, fibrin and inflammation scores are expressed as median (interquartile range).

GES, gallic acid-eluting stent; GSES, gallic acid and sirolimus-eluting stent; IEL, internal elastic lamina; NS, not significant; SES, sirolimus-eluting stent.

### Micro-CT analysis

Percent area of stenosis of the stented arteries detected using micro-CT was significantly lower in the GSES group compared to that in the SES group (Figure 4 and 5). Injury, fibrin, and inflammation scores were expressed as median (interquartile range).

**Figure 4.** Representative images of hematoxylin and eosin staining at 4 weeks after stenting. Specimens of implanted GES (A, ×20), GSES (B, ×20), and SES (C, ×20). Carstairs’ fibrin stain (magnitude, ×20) of fibrin infiltration in implanted GES (A-1, ×20), GSES (B-1, ×20), and SES (C-1, ×20). GES, gallic acid-eluting stent; GSES, gallic acid and sirolimus-eluting stent; SES, sirolimus-eluting stent.

**Figure 5.** Injury score (A), percent area of stenosis (B), fibrin score (C), and inflammation score (D) of GES, GSES, and SES. A, C, and D are expressed as the median (interquartile range). GES, gallic acid-eluting stent; GSES, gallic acid and sirolimus-eluting stent; SES, sirolimus-eluting stent.
and SES groups than in the GES group (45.4 ± 9.48% in GES vs. 40.9 ± 4.94% in GSES vs. 39.8 ± 9.49% in SES, p < 0.05) (Figure 6).

DISCUSSION

The present study was conducted to investigate the anti-inflammatory and anti-smooth muscle proliferative effects of gallic acid-eluting stents with/without sirolimus compared with sirolimus-eluting stents in a porcine coronary restenosis model. The results showed that gallic acid had a mild suppressive effect on vascular inflammation in the stented arteries. In addition, the injury score results showed that adequate pressure was applied according to the variable artery size.

The unpaired Student’s t test revealed no statistically significant difference in percent area stenosis between the SES and GSES groups (Figures 4 and 5). Conversely, the sirolimus-containing groups (SESs and GSESs) demonstrated significantly decreased neointimal hyperplasia compared to the GESs without sirolimus. Although the inflammation score was significantly lower in the two groups with gallic acid (GESs and GSESs), it was too weak to inhibit neointimal proliferation.

Macrocyclic immunosuppressive drugs such as sirolimus (rapamycin) bind to immunophilins to exert immunosuppressive effects. Sirolimus blocks the G1 to S cell cycle by interacting with mammalian target of rapamycin (mTOR) protein. This mechanism of action has been associated with inhibition of VSMC migration and proliferation after stenting. 32

Previous small and large animal studies reported that sirolimus could inhibit mammalian VSMC proliferation, and that its systemic administration could significantly reduce neointimal hyperplasia in a porcine coronary angioplasty model.19,33,34 Thus, first generation DESs used sirolimus to prevent the proliferation of VSMCs after percutaneous coronary interventions. Coronary SESs have also been shown to significantly reduce target lesion revascularization compared with BMSs in long-term follow-up in patients with acute myocardial infarction. 35

There have been several attempts to use natural products such as phytochemicals (phytoncides), paclitaxel,
and artemisinin derivatives to coat coronary stents to prevent the side effects of other synthetically coated drugs and polymers. The success of natural product-eluting stents has demonstrated the possibility of using natural products in coronary stents. Based on these previous reports, a new candidate for natural products was found.

Gallic acid (trihydroxybenzoic acid) is a phenolic acid and phytochemical which is found in sumac, witch hazel, oak bark, tea leaves, grapes, blackberries, gallnuts, and many plants. It has antioxidant, antimicrobial, and anti-inflammatory effects.

In a previous study in which gallic acid was coated on a metal plate to evaluate the inhibitory effect on human umbilical artery smooth muscle cell adhesion and proliferation, the coated surface showed remarkable inhibitory activity. In the present study, gallic acid exhibited insufficient suppression of neointimal proliferation, although the anti-inflammatory effect of gallic acid was noted. The results of this study suggest the feasibility of using natural substances to coat coronary stents. In our future research, stents using other natural polymers such as dextran or without polymers are under development.

CONCLUSIONS

Gallic acid added to coronary stents (GESs, GSESs) showed very mild anti-inflammatory effects at 1 month compared with SESs in a porcine coronary restenosis model. Although gallic acid itself did not show a sufficient effect on neointimal hyperplasia, the application of natural products to coronary stents has shown potential for use in other surface-coated medical devices.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

The authors and CG Bio. Co. Ltd have no conflicts of interest relevant to this article to report. CG Bio. Co. Ltd only served to coat the coronary stent. There was no funding offered.

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