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Drug-Eluting Stent for Delivery of Signal Pathway-Specific 1,3-Dipropyl-8-cyclopentyl Xanthine

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Abstract: 1,3-Dipropyl-8-cyclopentyl xanthine (DPCPX) is a highly selective antagonist of the adenosine A_1 receptor (A_1R). The A_1R mediates mitogenic effects of adenosine in coronary artery smooth muscle cells (CASMC). DPCPX plays a role as an antimitogen and reduces CASMC proliferation by the blockage of A_1R. A drug-eluting stent (DES) loaded with DPCPX was prepared. The water solubility of DPCPX is 1.6 μg/mL at pH 3−9, and 38.1 ± 2.3 μg/mL at pH 11. A series of DPCPX-eluting stents were formulated in polyurethane (PU) films with different dose densities and film thicknesses. The release of DPCPX from the PU-coated stents was nearly linear. The release rate and duration were effectively controlled by adjusting the film thickness with the same drug concentration. The eluted DPCPX from the PU films was effective in preventing CASMC proliferation, regardless of stimulation by 2-chloro-*N*-6-cyclopentyladenosine (CCPA), a highly selective A_1R agonist. A_1R specific antagonist DPCPX was effective in preventing CASMC proliferation and holds great promise for intracoronary delivery from DESs to test the role of the A_1R signaling pathway for prevention of in-stent restenosis.

Keywords: 1,3-Dipropyl-8-cyclopentyl xanthine; drug eluting stent; restenosis; coronary artery smooth muscle cells; adenosine receptor

1. Introduction

The occlusive cardiovascular diseases, including hypertension, atherosclerosis and restenosis, are known to be closely coupled with proliferation and migration of coronary artery smooth muscle cells (CASMCs).[1,2] Treatments of restenosis deal with the temporal pathological processes which involve blood coagulation, inflammation, proliferation/migration of smooth muscle cells, and endothelialization.[3] Drug-eluting stents (DESs), composed of bare metal stents, polymer coatings and antiproliferative drugs, are widely used clinically to treat severe coronary atherosclerosis and have been shown to reduce in-stent restenosis. Current drugs used in DESs in the clinic mostly act by blocking cell cycling and cell division (e.g., paclitaxel, rapamycin).[4−9] The problem with these drugs is that they also nonspecifically act on other cell types and may delay the recovery of a denuded endothelial layer,

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which can lead to increased thrombosis. Thus, it is necessary to discover specific antiproliferative agents that act specifically on CASMCs.[10−12]

Adenosine receptors as G protein-coupled receptors are transmembrane proteins composed of seven α helical integral domain and extra/intracellular connecting loops. Four subtypes of adenosine receptors (A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R) have been cloned and pharmacologically characterized. Each subfamily of adenosine receptors is known to modulate physiological functions in the body, but the roles of individual subfamilies vary depending on the cell type and the animal.[13] For example, mitogenic actions of A_1R in mice are not known, but A_1R activation decreases coronary blood flow in mice.[14] To our knowledge A_1R expression or function has not been studied in human coronary artery. The role of A_1R in CASMC proliferation has been studied in the Ossabaw miniature swine model[15] which has metabolic syndrome and type 2 diabetes.[16] The main advantage of the Ossabaw miniature swine model is that simple diet induces atherosclerosis and hyperplasia in a local stent region, mimicking those in human patients. This makes it easy to study treatment of restenosis by using an A_1R antgagonist.

Previous studies indicate that porcine CASMCs express A_1, A_{2A}, A_3 and barely detectable levels of A_{2B} receptor mRNAs.[17] Adenosine receptors are also differentially expressed on the human endothelial cells. Adenosine A_{2A} receptors are mainly expressed, while A_1 and A_3 receptors are not.[18] The A_1R mediates mitogenic effects of adenosine in CASMC *in vitro* via activation of the ERK1/2, JNK, PI3K-AKT signaling pathways.[17,19] *In vivo* study shows that adenosine A_1 receptor was up-regulated in stent-induced neointimal hyperplasia in coronary artery of Ossabaw miniature swine.[15] Together with evidence of A_1R expression in cardiovascular region, various selective agonists and antagonists of adenosine receptors are available aiming at adenosine receptors as therapeutic targets.[13] Thus, these differential expressions of adenosine receptors on the specific cells and appropriate employment of antagonists/agonists can be a strategy for a treatment, satisfying the needs of tailored molecular medicine by targeting diseases at specific local regions.

In this study, we tested the hypothesis that the highly selective A_1R antagonist 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX) would be effectively eluted from polymers used on DES and would block CASMC proliferation. DPCPX prevents mitogenesis by blocking A_1R of CASMC *in vitro*.[15,20] Since DPCPX is expected to selectively inhibit CASMCs proliferation *in vivo*, it is a highly potential pharmacological agent for intracoronary delivery from DESs to prevent in-stent restenosis, in which CASMC proliferation plays a very important role. The physiochemical properties of DPCPX were examined for loading into a polymer layer of a stent and the drug release profiles were studied to understand the release mechanisms. DPCPX eluted from PU film was used to examine whether it was able to inhibit proliferation of the subcultured CASMCs from Ossabaw swine through blockage of adenosine A_1 receptor mediated signaling.

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2. Materials and Methods

2.1. Materials. 2-Chloro-*N*-6-cyclopentyladenosine (CCPA) and 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX) were purchased from TOCRIS (Ellisville, MO). Segmented polyurethane (PU, Cardiomat 610) was purchased from Polymer Technology Group, Inc. (Berkeley, CA). Collagen type I was purchased from Inamed (Purecol, Fremont, CA). Bare metal stents (BMSs) were custom designed and manufactured by Burpee materials technology (Eatontown, NJ).

2.2. Preparation of DESs. Series of DPCPX/PU solutions were prepared in tetrahydrofuran (THF) (total solid concentration: 0.5 w/v %). The ratio of DPCPX/PU was adjusted to 10, 20, and 30 wt %. The solution of DPCPX and PU was coated on our custom manufactured BMSs using an electrostatic spray method. A BMS has surface area of 51.69 mm^2, 8 mm in length and 4 mm of diameter upon expansion. A stent was mounted on a mandrel connected with a rotator and a transverse system. The electrostatic spray conditions were fixed: 0.02 mL/min of flow rate, 8.0−10.0 kV of high voltage, and 10 psi of air flow. The distance between the stent surface and the spray nozzle was set as 1.5 cm. The amount of drug/polymer coated on a stent was confirmed by weight measurement after drying residual solvent.

2.3. Solubility and Release Study. DPCPX (1 mg) was added to 1 mL of distilled water at various pH ranging from 3 to 11. pH of distilled water was adjusted with 0.01 N of HCl or 0.1 N of NaOH. Samples were vigorously stirred for 48 h at 120 rpm at 37 °C, and then filtered with a nylon syringe filter (pore size: 0.2 μm). The DPCPX release from the dilated DESs was performed in phosphate buffered saline (PBS) at 120 rpm at 37 °C. Medium (1 mL) from the sample was taken at predetermined time periods, and the remaining medium was aspirated to be replaced with 2 mL of fresh medium. Samples were analyzed for DPCPX solubility and released DPCPX using high performance liquid chromatography (HPLC) (Agilent 1100 series) with UV detection at 227 nm. Samples were run with 1 mL/min of flow rate at 25 °C. Methanol and water at the 6:4 ratio were used as a mobile phase.

2.4. Preparation of DPCPX-Eluting Films. DPCPX and PU solutions (10 wt %) were prepared in THF. The ratio of a drug to a polymer was varied from 0 wt % to 30 wt % of the total solid. The solution was dropped on the 15 mm round cover glass (Esco, Portmouth, NH) and was spin-coated at 1000 rpm. The coated films on the cover glass were dried under vacuum for 24 h to remove residual solvent. A drug-eluting film coated on the cover glass was placed in each well of a 24-well plate for the cell culture study.

2.5. Isolation and Culture of Porcine CASMC. Hearts were harvested from Ossabaw pigs. Coronary arteries dissected from the hearts were placed in a physiological buffer. Porcine CASMCs were enzymatically dispersed from coronary arteries using collagenase solutions as described.[17,19] Primary cultured CASMCs were subcultured at 37 °C with 5% CO_2 in DMEM containing 10% fetal bovine serum (FBS). CASMCs cultured with 80% confluence were used between passage 4 and 10.

2.6. *In Vitro* Evaluation of DPCPX Eluted from PU Film on CASMC Proliferation. The CASMCs were seeded on the surface of type I collagen that was gelated on the DPCPX-loaded PU film. The DPCPX-loaded PU films with various drug/polymer ratios and pure PU film as a control were placed in 24-well plates. Collagen solution was prepared by mixing eight parts of PureCol (3 mg/mL) with one part of 10X PBS. The pH of the collagen solution was adjusted to 7.4 by adding 0.01 N HCl and 0.1 M NaOH. Collagen solution (300 μL) was added to each well containing the PU/DPCPX-coated cover glass. After 30 min of incubation at 37 °C, collagen matrix/DPCPX-loaded PU film was washed with PBS and filled with DMEM containing 10% FBS. Subculture CASMCs were suspended by trypsinization, counted and seeded on the collagen matrix/DPCPX-loaded PU film in 24 wells. Seeding density was 4,000 cells per well in DMEM containing 10% FBS in a 24-well plate. CASMCs were stimulated in the presence of CCPA for 2 days. After 4 days, cell proliferation was estimated using an MTT cell growth assay kit (Chemicon international, Temecula, CA). The tetrazolium compound 3-(4,5-dimehtylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 20 μL) was added to 100 μL of fresh DMEM. The CASMCs were incubated in the solution for 4 h at 37 °C. The absorbance was read at 490 nm, which was directly proportional to the number of living cells.[17,19] The measured absorbance was compared with the control in percent.

2.7. Scanning Electron Microscope (SEM). The morphology and coating integrity of a DPCPX and PU coated stent were characterized using scanning electron microscopy (JEOL JSM-840; JEOL USA; Peabody, MA). Stents were mounted to aluminum stubs and coated with gold palladium in argon gas using a sputter coater (Hummer I; Anatech Ltd.; Hayward, CA). Coated stents were observed and imaged using SEM with an accelerating voltage of 4 kV, a probe current of 3×10^{-11} A, and a working distance of 15 mm.

2.8. Fluorescence Confocal Laser Scanning Microscopy (CLSM). Cells were fixed with 4% formalin and stained with propidium iodide solution. Stained CASMCs were observed with fluorescence laser scanning confocal microscopy (model MRC-1024, Bio-Rad, Hercules, CA) equipped with a krypton/argon laser and a Nikon Diaphot 300 inverted microscope.

3. Results and Discussion

3.1. Solubility of DPCPX. The chemical structure and pharmacological mechanism of DPCPX are shown in Figure 1-A. DPCPX, an A_1 AR antagonist, is the modified structure of the xanthines by a cyclopentyl derivative group.[13] For the release study, the solubility of DPCPX was measured in water, PBS (pH 7.0), and PBS/0.05 wt % Tween 20 (pH 7.0). DPCPX (1 mg) was added to 1 mL of each medium and stirred for 48 h at 37 °C. The solubility of DPCPX measured in water, PBS, and PBS/0.05% Tween 20 at pH

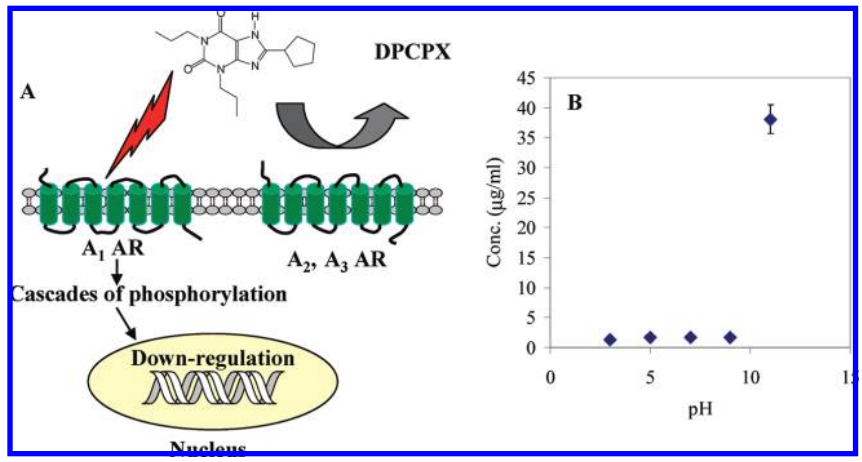

Figure 1. Chemical structure and pharmacological action of 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX) (A), and the pH-dependence solubility of DPCPX in water (B).

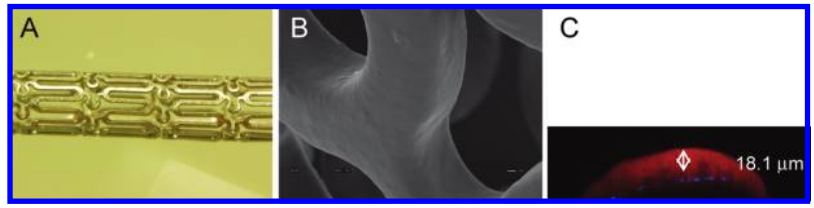

Figure 2. Bright field microscopy (A), SEM (B), and CLSM (C) images of DPCPX-eluting stents. For the orthogonal image taken by CLSM, the PU film was stained with nile red.

7.4 was 1.62 ± 0.12, 1.69 ± 0.72, and 1.63 ± 0.08 μg/mL, respectively. There were no statistical differences among the solubilities. The solubility of DPCPX was also measured as a function of pH in water, ranging from 3 to 11, as shown in Figure 1-B. The DPCPX solubility (approximately 1.6 μg/mL) in water was not significantly changed between pH 3 and 9, while it reached 38.1 ± 2.3 μg/mL at pH 11. The solubility of DPCPX in water at pH 11 was 28 times higher than the solubility at pH 3−9. If such a high solubility increase occurred at pH closer to the physiological pH, the aqueous solution at that pH could have been used as a release medium providing a sink condition. Unfortunately, pH 11 was too far away from the physiological pH, and thus, the subsequent *in vitro* release experiments of DPCPX-eluting stent were carried out in PBS at pH 7.4.

Custom-made BMSs were coated with DPCPX/PU using an electrospray coating method. The solid concentration of the polymer and the drug in spraying solution was 0.5 wt % in THF, and the ratio of drug to polymer was varied from 10 to 30 wt %. The density of DPCPX per stent (μg/mm^2) was varied by changing the drug/PU ratio in the spraying solution and the spraying time. The amount of coated polymer/drug was calculated by measuring the weight of stents before and after coating to obtain the desired drug density per stent.

Images of DPCPX-eluting stents were taken with bright-field microscopy, SEM, and CLSM (Figure 2). DPCPX-eluting stents coated with PU had no web formation along the wire and the smooth surface texture of the PU film was observed, as shown in panels A and B in Figure 2. After expansion of a DPCPX-coated stent, no delamination or peeling-off of the PU film was observed, indicating that a DPCPX/PU coated stent can withstand the compressibility and the strain associated with expansion of the stent. The SEM image indicated that the film surface was smooth and no drug crystals were formed, indicating that DPCPX was well integrated in the PU matrix without separation.

Table 1. The Thickness of the Films Prepared under Different Coating Conditions

	drug concn % (w/w)	av thickness (μm)	drug density (μg/mm^2)
10%-0.5	10	19.2 ± 0.8	0.5
10%-1	10	23.5 ± 0.9	1
10%-2	10	37.6 ± 1.4	2
20%-1	20	14.9 ± 1.7	1
30%-1	30	6.9 ± 0.5	1

The thickness of the PU film coated on a BMS was measured by staining the film with 0.01 wt/v % of nile red in water. The orthogonal images of a stent strut were taken with CLSM, as shown in Figure 2-C. The variance of film thicknesses is presented in Table 1, as DPCPX-eluting stents were coated under different conditions. A schematic representation of the targeting dose density and the resulting film thickness is presented in Figure 3. The first and the second numbers in the notations in Table 1 and Figure 3 represent the drug/PU ratio in the spraying solution and the drug surface density, respectively. For example, 10%-1 indicates the DPCPX concentration of 10 wt % and the drug surface density of 1 μg/mm^2. A series of stents (set A) were prepared with varied drug/PU ratios (10, 20, and 30 wt %) at the constant drug surface density of 1 μg/mm^2. The polymer

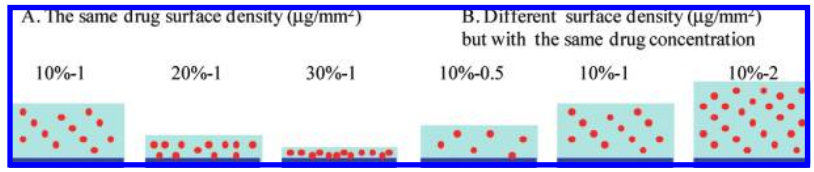

Figure 3. Schematic presentation of the coating condition and expected film thickness. (A) Constant drug dose density using different drug concentrations in coating solution. (B) Constant drug concentration in coating solution with different drug dose densities. The first number presents the drug composition of a sprayed solution, and the second number is the targeted drug surface density.

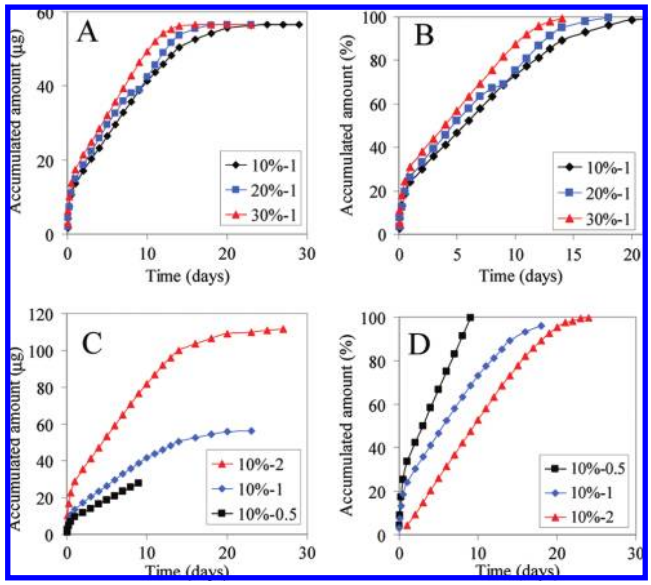

Figure 4. DPCPX release profiles in the cumulated total amount (A and C) and in the percent release (B and D) from different formulations. The stents were coated with solutions of different concentrations for the same drug surface density (A and B). The stent coated with varied drug surface density using the same 10% DPCPX spraying solution (C and D).

thicknesses of 10%-1, 20%-1, and 30%-1 stents were 23.5 ± 0.9, 14.9 ± 1.7, and 6.9 ± 0.5 μm, respectively. The film thickness was decreased with the increase in the drug/PU ratio of the spraying solution, because the total drug density was maintained constant for each stent. Another series of stents (set B) were coated with the spraying solution with 10 wt % DPCPX/PU ratio and the drug surface density was varied as 0.5, 1, and 2 μg/mm^2 by adjusting the duration of spray coating. The measured thicknesses of 10%-0.5, 10%-1 and 10%-2 were 19.2 ± 0.8, 23.5 ± 0.9, and 37.9 ± 1.4 μm, respectively. The film thickness was increased with the increase of the drug density at the constant DPCPX/PU ratio in the spraying solution.

3.2. DPCPX Release Profiles. The drug elution profiles of the two different sets of PU films were examined instead of expanded state of stents. As shown in Figure 4, for all PU-coated stents, DPCPX release was nearly linear after the initial burst release on the first day. The release rate was slightly accelerated with the increase in the drug/PU ratio on the stent coated at the constant drug density of 1 μg/mm^2 (30% > 20% > 10% DPCPX/PU ratio). The ratio between the drug and PU in the spraying solution, however, did not change the extent of release rate significantly (Figure 4-A,B), as compared with paclitaxel release from the poly(styrene-*b*-isobutylene-*b*-styrene) (SIBS) matrix for the same formulation condition in previous study.[5] The periods of DPCPX release were almost the same for the formulations in the range of 14 days.[8]

For the second set of release study, the duration of DPCPX release was prolonged, as the total drug amount loaded on the stent and the drug density were increased at the constant drug/PU ratio (10 wt %). The durations of release were 9 days for 10%-0.5, 23 days for 10%-1, and 28 days for 10%-2, respectively (Figure 4-C,D). The drug release rate was also accelerated with the increase of the drug surface density at the constant drug/PU ratio (10 wt %) of the spraying solution. As the total drug amount or the drug surface density was increased, the film on the stent became thicker. The increased film thickness resulted in drug diffusion for longer distance and thus for longer period of time. Since only the duration of spraying time was adjusted, using the spraying solution with the constant drug/PU ratio (10 wt %), the drug concentration (i.e., volume density) is similar in all three cases (10%-0.5, 10%-1, and 10%-2), and drug distribution is uniform throughout the film thickness. Thus, the film thickness and the total drug amount per stent were the major components to modulate the drug release from the DPCPX/PU-coated stents, because release was dominated by diffusion through the polymer film.

3.3. Effect of DPCPX Eluted from PU Film on the Porcine CASMC *in Vitro*. The signal pathways relevant to adenosine-induced CASMC mitogenesis have been studied using various pharmacological agents.[15,18,19,21] Stimulation of CASMCs by adenosine and other agonists increases the phosphorylation levels of extracellular signal regulated kinase 1/2 (ERK1/2), jun N-terminal kinase 1/2 (JNK1/2) and PI3K-AKT in a dose-dependent manner, indicating that adenosine activates the ERK, JNK and PI3K-AKT signaling pathways. CCPA, an A_1R selective agonist, has been a useful tool to study adenosine activation of mitogenic signaling pathways. Both adenosine and CCPA increase DNA synthesis, protein synthesis, and cell number in porcine CASMC.[17,19] In contrast, DPCPX, an A_1R selective antagonist, significantly

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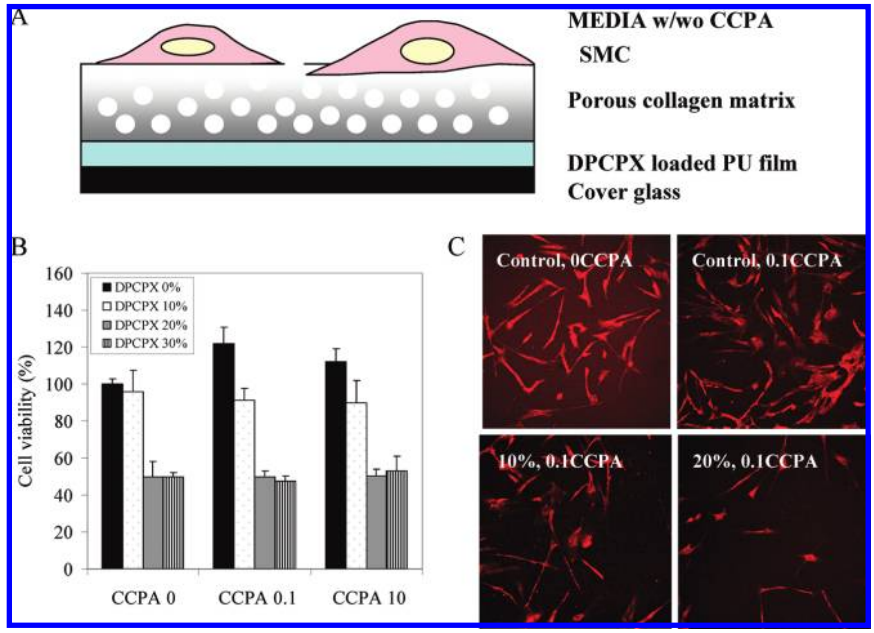

Figure 5. Effect of eluted DPCPX on CASMC *in vitro.* (A) Schematic description of a porous collagen matrix placed on top of the PU film. (B) SMC viability analyzed by MTT assay in the presence of different CCPA concentrations. (C) Growth of SMCs in response to DPCPX stimulation at various CCPA concentrations. Images were taken by fluorescence confocal laser scanning microscopy. SMC were fixed with formalin and stained with propidium idiode.

decreases adenosine-induced ERK, JNK, and AKT phosphorylation in a dose-dependent manner.

The effects of DPCPX released from PU films on the CASMC proliferation *in vitro* were evaluated to find an optimum condition for developing a DPCPX-eluting stent. For the cell culture study, DPCPX-loaded PU films were casted on cover glass by spin coating. The drug amount in the PU film on each cover glass was 9.8 ± 1.36 μg for 10%, 25.8 ± 0.78 μg for 20%, and 52.1 ± 0.94 μg for 30% DPCPX, respectively. CASMCs did not grow on the PU film, and thus, a porous collagen gel matrix was placed on the DPCPX-loaded PU film. Collagen matrix was 300 μm in thickness as measured from autofluorescence of an orthogonal image of collagen matrix taken with CLSM. Then, SMCs were seeded on the collagen gel matrix. The highly porous collagen matrix does not impede the drug diffusion to medium.[22] CASMCs attached on the collagen gel matrix and proliferated. SMCs are embedded in the extracellular matrix in the coronary artery, and a drug from a stent needs to diffuse though the extracellular matrix to affect the SMC proliferation. Thus, our experimental design using DPCPX-loaded PU films coated with collagen gel matrix can be considered to mimic the physiological condition for testing the effect of DPCPX elution on CASMCs.

CASMC antiproliferation effects of DPCPX in the absence or presence of CCPA were examined by monitoring cellular DNA synthesis via [^{3}H]thymidine uptake, protein synthesis via [^{3}H]leucine, and consequent live cell number via MTT assays in our previous study.[17,19] Since the antiproliferation effect of DPCPX at the molecular level was already proven in CASMC culture *in vitro*, we have examined the only live cell number via MTT assay. CASMCs were exposed to CCPA at different concentrations (0, 0.1, 10 μM) for the first 2 days. After 2 days, CCPA was removed by replacing the medium with fresh medium without CCPA. At the same time, DPCPX as an A_1R specific antagonist was competitively exposed to CASMCs by diffusion from the PU film through a collagen gel layer (Figure 5-A). The control with no CCPA exposure in medium and no DPCPX diffusion from the PU film reached 80% confluence of CASMC at day 4. Thus, the effects of DPCPX on CASMC proliferation were evaluated by CASMC proliferation at day 4 using MTT assay, as shown in Figure 5-B. The other control with CCPA exposure and no DPCPX release showed increased CASMC viability (121.8% at 0.1 μM CCPA and 112.2% at 10 μM CCPA), as compared with the control (100%, no CCPA, no DPCPX). The increased cell viability in the presence of CCPA indicates that cell proliferation was augmented by increased DNA and protein synthesis that were stimulated by CCPA via the activation of A_1R receptor. At 10% DPCPX loading, viability was 95.9% at 0 μM CCPA, 91.23% at 0.1 μM CCPA, and 89.6% at 10 μM CCPA, These values are not much different from the cell viability of the control (100.0%, no DPCPX, no CCPA). CASMC viability was not changed significantly, regardless of the CCPA concentration, compared with the control (no CCPA, no DPCPX). The total drug amount of 10% DPCPX-loaded film on the glass was only 9.8 μg in each well, which is 5.9 times lower than that total amount DPCPX of the 10% loaded stent. The majority

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of DPCPX might have been depleted during the initial burst in the first few days. Thus, after refreshing the medium without CCPA, the drug amount released from 10% DPCPX-loaded PU film might not have been sufficient to affect cell proliferation of CASMC. In contrast, the competitive exposure of DPCPX in the presence of 0.1 μM CCPA in the medium resulted in reduced CASMC proliferation, showing that viability was 25% lower than that by only CCPA stimulation (0.1 μM CCPA, no DPCPX). This result indicates that DPCPX behaved as an effective antagonist of A_1R receptor, competitively acting for adenosine receptor against CCPA.

DPCPX release from the 20% and 30% DPCPX-loaded films without exposure of CCPA showed that cell viability was significantly decreased to 49.8%, compared with the control (no CCPA, 10% DPCPX). In addition, exposure of 0.1 μM and 10 μM CCPA agonist did not alter the effect of DPCPX, showing the reduced cell viability of approximate 50%. This result is coincident with that CCPA-induced DNA and protein synthesis were dramatically inhibited in a concentration-dependent manner by DPCPX as previously reported in the literature.[17,19] DPCPX released from the PU film was effective in reducing CASMC proliferation and viability. Competitive exposure with the A_1R agonist, CCPA, confirms the selective effect of DPCPX antagonism of the A_1R. The cell morphology and confluence were observed by staining CASMCs with propidium iodide, as shown in Figure 5-C. Spindle shape of SMC was maintained in the presence of CCPA/DPCPX drugs. The confluence was affected by addition of an agonist or an antagonist. The SMC growth was enhanced in the presence of CCPA without DPCPX, whereas confluence of SMC was reduced in presence of CCPA/DPCPX. The presence of DPCPX successfully diminished the SMC proliferation in vitro even in the presence of CCPA. The images showed the similar confluence as MTT assay of cell viability.

The DPCPX/PU films for *in vitro* cellular experiments were coated by spin-coating. Even though the composition of drug/polymer is the same as that of the stent-coated films, the coated films of 1−3 μm in thickness are thinner, since the excess of drug/polymer solution was removed during the coating process due to centrifugal force. In contrast, stent coated by electrospray was coated with fine mists for 5−10 min. Thus, drug distribution through the film in depth might be different, resulting in no comparable release profiles between stent and glass coating. However, the *in vitro* cellular study is still highly meaningful in that the varied DPCPX released amounts affected cellular growth rate. The release profiles from the drug-eluting stents will be more relevant to the intervention of coronary artery in the *in vivo* studies of Ossabaw swine model in the future.

3.4. Discussion. Adenosine receptors have been studied as therapeutic targets of various diseases, such as arrhythmias, ischemia, neurodegenerative diseases, sleep disorders, diabetes, and cancer.[13] Various selective agonists and antagonists of different adenosine receptor subtypes have been discovered. It is promising to treat many diseases by judicious selection of the right agonists or antagonists depending on differential expression of the adenosine receptor subtypes. We have previously reported that adenosine A_1 receptor was upregulated in the region of stent-induced neointima of Ossabaw miniature swine.[23] It was also reported that A_{2B} deficiency enhanced postinjury neointima formation in the vasculature in A_{2B} receptor knockout mice. A_{2B} receptor is known to be involved in protection against SMC proliferation in neointima formation,[24] and mediates antimitogenesis in aortic vascular SMC in rat.[25] Although A_1R attenuates coronary blood flow in A_1R knockout mice, the mitogenic role was not determined.[17] The hypothesis in this study is that A_1R mediates mitogenesis in CASMCs. The functions of individual adenosine receptors in a local region of SMC proliferation vs antiproliferation can be highly dependent on the balance between the expression level and signaling efficiency of these receptors. The evidence of elevated A_1R expression in local stent region[15] provides a new possibility of using drugs specific to the local cellular signal pathway for reducing SMC proliferation in the stent region. The Ossabaw miniature swine model is a useful tool for studying such drugs that are released from the drug-eluting stents.

Stenting in the coronary artery increased expression of A_1 receptor, as compared with the nonstented coronary artery. Released DPCPX successfully prevented SMC proliferation even in the presence of CCPA, an agonist of A_1 receptor. Thus, use of DPCPX as a drug to prevent in-stent restenosis may be a valuable approach. It was demonstrated that adenosine A_1 receptor antagonist DPCPX released from a stent as a therapeutic agent has a potential to prevent proliferation of CASMC.

Bare metal stents are deployed in coronary arteries at the site of severe, blood-flow limiting atherosclerotic lesions. However, renarrowing and blockage, i.e. in-stent restenosis, can occur mainly due to CASMC proliferation. Drug-eluting stents (local drug delivery from a stent) have been used to prevent in-stent restenosis. For the clinically used DESs, CASMC proliferation is controlled by delivery of either paclitaxel, an anticancer agent, or rapamycin, an immunosuppressive drug. Both drugs work well for prevention of CASMC hyperplasia, but they also can prevent growth of endothelial cells, as their pharmacological effects are non-

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specific.[26] Growth of endothelial cells over the stent area is essential for long-term patency of the DES. For this reason, the discovery of a CASMC-specific antiproliferative agent for DESs may be a very important advance in interventional cardiology. In this study, DPCPX was used as a model agent in DESs, which acts on the A_1 receptor that is differentially expressed on porcine coronary artery endothelial cells and SMCs.

The efficacy of DPCPX itself has been shown in our previous study that CASMC proliferation was prevented by DPCPX.[15,19] In this study, DPCPX was successfully formulated within PU polymer matrix without disintegration of drug from the polymer matrix. Smooth integration of drug was observed from SEM pictures. Release profiles from DPCPX-eluting stents showed sustained release of DPCPX. In the drug release study, 2 mL of PBS buffer was used as a release medium which was replaced with a fresh solution every day. Previous studies used 1.5 mL of medium with addition of 10% ethanol or 1.5 mL of PBS with 0.005% Tween 20 per stent.[5,27] Addition of ethanol in the release medium removed solubility limitation, but ethanol affected the release profiles as confirmed in the accelerated release studies. For this reason, we did not use ethanol in this study. It is possible that the release profile may have affected partially by the dissolution-limited release, in addition to diffusion-limited release. But we decided to use the PBS buffer without any organic solvent to avoid potential artifact that may result from the presence of an organic solvent.

4. Conclusion

DPCPX, a signal pathway specific drug, was examined to develop a DES. DPCPX-eluting stents with different drug dose densities and thicknesses were formulated for tailored drug-eluting profiles. The duration of drug release and release rate were efficiently modulated by adjusting the film thickness when the drug was uniformly distributed throughout the film. DPCPX eluted from DES polymer was effective in reducing CASMC proliferation by selective antagonism of the A_1R receptor. This study opens a new avenue to explore novel potential signal pathway specific drugs for developing new DESs that can selectively prevent the CASMC proliferation. We are currently preparing DPCPX-loaded stents for *in vivo* studies in the Ossabaw miniature swine model of metabolic syndrome and type 2 diabetes, which shows profound in-stent neointimal hyperplasia mimicking human patients having severe coronary restenosis.[10,15,16]

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