DOCTORAL THESIS

Antenatal administration of betamethasone contributes to
intimal thickening of the rat ductus arteriosus

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Antenatal Administration of Betamethasone Contributes to Intimal Thickening of the Rat Ductus Arteriosus

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**Background:** Antenatal betamethasone (BMZ) is a standard therapy for reducing respiratory distress syndrome in preterm infants. Recently, some reports have indicated that BMZ promotes ductus arteriosus (DA) closure. DA closure requires morphological remodeling; that is, intimal thickening (IT) formation; however, the role of BMZ in IT formation has not yet been reported.

**Methods and Results:** First, DNA microarray analysis using smooth muscle cells (SMCs) of rat preterm DA on gestational day 20 (pDASMCs) stimulated with BMZ was performed. Among 58,717 probe sets, ADP-ribosyltransferase 3 (Art3) was markedly increased by BMZ stimulation. Quantitative reverse transcription polymerase chain reaction (RT-PCR) confirmed the BMZ-induced increase of Art3 in pDASMCs, but not in aortic SMCs. Immunocytochemistry showed that BMZ stimulation increased lamellipodia formation. BMZ significantly increased total paxillin protein expression and the ratio of phosphorylated to total paxillin. A scratch assay demonstrated that BMZ stimulation promoted pDASMCL migration, which was attenuated by Art3-targeted siRNAs transfection. pDASMC proliferation was not promoted by BMZ, which was analyzed by a 5-bromo-2-deoxyuridine (BrdU) assay. Whether BMZ increased IT formation in vivo was examined. BMZ or saline was administered intravenously to maternal rats on gestational days 18 and 19, and DA tissues were obtained on gestational day 20. The ratio of IT to tunica media was significantly higher in the BMZ-treated group.

**Conclusions:** These data suggest that antenatal BMZ administration promotes DA IT through Art3-mediated DASMC migration.

**Key Words:** ADP-ribosyltransferase 3 (Art3); Ductus arteriosus; Glucocorticoid; Intimal thickening

The ductus arteriosus (DA) is a fetal arterial connection between the pulmonary arteries and the aorta, and it is indispensable for maintaining fetal life. In full-term infants, this fetal structure normally closes during the first few days after birth. In preterm infants, however, the DA often remains patent after the neonatal period; this condition is termed persistent patency of the DA (PDA).1 PDA is a major cause of morbidity and mortality with systemic hypo-perfusion and pulmonary congestion.2,3 Although more than 50% of infants with birth weights <1,000 g receive pharmacological therapy with cyclooxygenase inhibitors or surgical ligation of the DA, the current strategies for PDA are not always ideal due to their invasiveness.4 Therefore, further investigation of molecular mechanisms of regulating DA is important.

Maternal administration of betamethasone (BMZ) is an effective therapy for reducing respiratory distress syndrome (RDS) and improves morbidity and mortality in preterm infants.5,6 RDS is a severe complication and a poor prognostic factor of preterm infants, and antenatal BMZ treatment aims for fetal lung maturation and is currently a standard form of care in cases of imminent or anticipated preterm delivery.5,6 In addition, observational studies have demonstrated that the prevalence of PDA is lower in human preterm infants who received antenatal steroid therapy.7-12 The contractile effect of glucocorticoids on the DA has been studied in preterm lamb,13 term rat,14 preterm mouse,15 and preterm human fetuses.16,17 A study using mouse DA rings demonstrated that BMZ exposure increased the sensitivity of the DA to oxygen, which was accompanied by an expression change of some genes that mediate oxygen-induced constriction and vasodilation.18 Together with previous observational studies, these data suggest that antenatal BMZ promotes DA contraction. In addition to muscle contraction, morphological remodeling, such as intimal thickening (IT) formation, is required.
Betamethasone Promotes DA Intimal Thickening

for achieving anatomical closure of the DA.\textsuperscript{1,18-23} In humans, IT gradually develops during the fetal period. There are several sequential processes of IT formation in the DA. Subendothelial edema and disruption of the internal elastic lamina starts at \textasciitilde17 weeks gestation in humans,\textsuperscript{1} and subsequent vascular smooth muscle cell (SMC) migration into the subendothelial region becomes prominent from the mid- to late-gestational periods.\textsuperscript{1} Hence, IT formation is not fully developed in preterm infants.\textsuperscript{23,24} Glucocorticoid is known to promote migration in several types of cells, including neuronal cells, cancer cells, and immune cells.\textsuperscript{25-27} However, the role of glucocorticoid in vascular SMC migration and IT formation of the DA remains unknown.

Here, we examined the effect of BMZ on gene expression changes in rat preterm DA SMCs (pDASMCs), SMC migration, and IT formation in vivo.

**Methods**

**Reagents**

Betamethasone sodium phosphate, \(\beta\)-mercaptoethanol, and buffered formalin were purchased from Wako (Osaka, Japan). Collagenase dispase was purchased from Roche (Tokyo, Japan). Collagenase II was purchased from Worthington Biochemical (Lakewood, NJ, USA). Fetal bovine serum (FBS) was purchased from Equitech-Bio (Kerrville, TX, USA). Elastase type II-A, trypsin inhibitor type I-S, poly-L-lysine, Hank's balanced salt solutions, Dulbecco's modified Eagle's medium (DMEM), bovine serum albumin (BSA), penicillin, streptomycin, and actinomycin D were purchased from Sigma (St. Louis, MO, USA). TRizol was purchased from Invitrogen (Carlsbad, CA, USA). DNase I was purchased from Promega (Tokyo, Japan). Anti-phosphorylated paxillin (Tyr\textsuperscript{189}) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Rhodamine conjugated anti-F-actin antibody, Alexa Flour 488 anti-rabbit IgG, and Hoechst 33342 were purchased from Life Technologies (Carlsbad). Anti-paxillin was purchased from BD Biosciences (San Jose, CA, USA). Anti-GAPDH was purchased from Santa Cruz (Dallas, TX, USA).

**Animal Studies**

Wistar rat fetuses were obtained from timed-pregnant rats that were purchased from Japan SLC, Inc. (Shizuoka, Japan). All animal studies were approved by the Institutional Animal Care and Use Committees of Yokohama City University in accordance with the Guide for the Care and Use of Laboratory Animals (reference number: F-A-16-010).

**Primary Culture of Rat Preterm DA and Aorta SMCs**

Vascular SMCs in primary culture were obtained from the DA and the aorta of Wistar rats on gestational day 20 (pDASMCs and pASMCs, respectively). Gestational day 18–20 is equivalent to the second trimester pregnancy in humans.\textsuperscript{28} Isolation of pDASMCs and pASMCs was performed using the same procedure as described previously.\textsuperscript{28} Briefly, the fetal vascular tissues were transferred to a 1.5-mL centrifuge tube that contained 800\,\mu L of enzyme mixture (1.5\,mg/mL collagenase-dispase, 0.5\,mg/mL of elastase type II-A, 1\,mg/mL of trypsin inhibitor type I-S, and 2\,mg/mL of bovine serum albumin fraction V in Hank's balanced salt solutions). Digestion was carried out at 37°C for 15 min. Cell suspensions were then centrifuged, and the medium was changed to a collagenase II enzyme mixture (1\,mg/mL collagenase II, 0.3\,mg/mL trypsin inhibitor type I-S, 2\,mg/mL of bovine serum albumin fraction V in Hank's balanced salt solutions). After 12 min of incubation at 37°C, cell suspensions were transferred to a growth medium in 35-mm poly-L-lysine-coated dishes in a moist tissue culture incubator at 37°C in 5% CO\textsubscript{2}–95% ambient mixed air. The growth medium contained DMEM with 10% FBS, 100\,\mu M penicillin, and 100\,\mu g/mL streptomycin. The confluent cells were used at passages 3–5. We confirmed that >99% of cells were positive for \(\alpha\)-smooth muscle actin and showed the typical "hill-and-valley" morphology.

**DNA Microarray Analysis**

The pDASMCs were stimulated for 24\,h with or without BMZ (100\,
\mu M/L, \(n=2\). Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA was converted to cyanine3-labeled cRNA that was hybridized to SurePrint G3 Human GE 6x60K v2 Microarray (Agilent, Santa Clara, CA, USA) for 17\,h at 65°C. The hybridization signals on the microarray were scanned and analyzed using a Gene-Array Scanner and Microarray Suite Software (Agilent). Of the 58,717 probe sets on the microarray, we analyzed their differential expression between BMZ and the control. We selected the genes that had more than a 3.0-fold change.

**RNA Isolation From SMCs**

Both pDASMCs and pASMCs were stimulated with or without BMZ (1, 10, or 100\,\mu M/L) for 24\,h. For the time-course study, pDASMCs were stimulated with BMZ (100\,\mu M/L) combined with or without actinomycin D (5\,\mu g/mL). Total RNA was isolated using an RNeasy Mini Kit according to the manufacturer's instructions.

**Quantitative RT-PCR**

Reverse transcription-polymerase chain reaction (RT-PCR) was performed as described previously.\textsuperscript{30} The primers were designed between multiple exons based on the rat nucleotide sequences of Ar3 and Nr3c1 (Glucocorticoid Receptor) (5'–CCCTCTTCGGAGATGAAA–3' and 5'–CACGACGACGCGATGAGTAAA–3' for Ar3, and 5'–GGACACGCTGACCTTCTTG–3' and 5'–CCTATCGGAGCACACCA–3' for Nr3c1). The abundance of each gene was determined relative to 18S rRNA.

**Immunoblot Analysis**

pDASMCs cultured on 6-well culture plates were serum starved for 24\,h and then stimulated for 24\,h with or without BMZ (100\,\mu M/L). Cells were prepared in a cell lysis buffer (150\,mM/L NaCl, pH 11.0, 1\,mM/L EDTA2Na) and homogenized by sonication. Proteins from whole cells were analyzed by immunoblotting as described previously. Briefly, proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride membrane by electroblotting. Membranes were blocked in TBS-0.1% Tween containing 3% BSA and incubated with anti-phosphorylated paxillin antibody or anti-paxillin antibody overnight at 4°C. Specific binding was visualized by the Western Lighting ECL Pro (PerkinElmer, Waltham, MA, USA).
Figure 1. Effect of betamethasone (BMZ) on mRNA expressions of ADP-ribosyltransferase 3 (Art3) and glucocorticoid receptor (GR) in preterm ductus arteriosus smooth muscle cells (pDASMCs). (A) Art3 mRNA expression in rat pDASMCs and pre-term aorta smooth muscle cells (pASMCs) was quantified by real-time reverse transcription polymerase chain reaction (RT-PCR). n=6, *P<0.05. (B) Art3 mRNA expression in pDASMCs treated with or without actinomycin D (1 μg/mL). n=6, *P<0.05. (C) GR (NAdc-7) mRNA expression in pDASMCs and pASMCs. n=6. NS, not significant.

Figure 2. Effect of betamethasone (BMZ) stimulation on preterm ductus arteriosus smooth muscle cells (pDASMCs) migration and proliferation. (A) Art3 mRNA expression in rat pDASMCs cultured on 24-well culture plates were serum starved by DMEM containing 0.1% FBS for 24 h and then stimulated for 24 h in media alone (control) or BMZ (100 μmol/L). Cells were then fixed in 10% buffered formalin and subjected to immunocytochemistry. Fixed cells were incubated with anti-phosphorylated paxillin (Tyr118) and rhodamine conjugated anti-F-actin antibodies for 24 h at 4°C. After 3 washes with Tween 20/PBS, cells were incubated with a secondary antibody, Alexa Fluor 488 anti-rabbit IgG, for 1 h. After 6 washes with Tween 20/PBS, DNA was stained with Hoechst 33342 solution for 20 min. After 2 washes with PBS, coverslips were mounted for microscopic imaging. Morphometric analyses were performed using Nikon ECLIPSE Ti (Tokyo, Japan).

Immunocytochemistry and Evaluation of Lamellipodia Formation

Immunocytochemical analysis was performed as previously described. Briefly, pDASMCs cultured on 24-well culture plates were serum starved by DMEM containing 0.1% FBS for 24 h and then stimulated for 24 h in media alone (control) or BMZ (100 μmol/L). Cells were then fixed in 10% buffered formalin and subjected to immunocytochemistry. Fixed cells were incubated with anti-phosphorylated paxillin (Tyr118) and rhodamine conjugated anti-F-actin antibodies for 24 h at 4°C. After 3 washes with Tween 20/PBS, cells were incubated with a secondary antibody, Alexa Fluor 488 anti-rabbit IgG, for 1 h. After 6 washes with Tween 20/PBS, DNA was stained with Hoechst 33342 solution for 20 min. After 2 washes with PBS, coverslips were mounted for microscopic imaging. Morphometric analyses were performed using Nikon ECLIPSE Ti (Tokyo, Japan).

Lamellipodia formation was evaluated morphologically using immunocytochemistry. pDASMCs showing lamel-
lipodial formation accompanied by phosphorylated paxillin were counted as positive. Lamellipodia-positive cell rate was calculated as the ratio of the positive cell number to the total cell number. The average of 20 fields was used for the value for each sample.

RNA Interference and Migration Assay
pDASMCs plated on 3.5-cm cell culture dishes were maintained in DMEM containing 10% FBS. Small interfering RNAs (siRNA) of Ar3 (siAr3-1; s156699, 5'-GGACAGUCGAACGCUGAATTT-3' and 5'-UUUCGAGCUUCUGACUCCAG-3', and siAr3-2; s156611, 5'-GACGAAUAAUGGAUGCCATT-3' and 5'-UGACACUUCAAUAUUCGUCGT-3') and control siRNA were obtained from Applied Biosystems (Foster City, CA, USA) and Qiagen respectively. According to the manufacturer's.
Table. BMZ-Induced Genes and Rn18s in pDASMCs

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BMZ, betamethasone; pDASMCs, preterm ductus arteriosus smooth muscle cells.

instructions, pDASMCs were transfected with Art3-targeted siRNA (75 pmol/well) or control siRNA using Lipofectamine RNAiMAX (Invitrogen). Twenty-four hours after transfection, cells were serum starved by DMEM containing 0.1% FBS for 24h, followed by treatment with or without 100 µmol/L BMZ for 72h. Cell migration was evaluated by measurement of the migration area of pDASMCs using Nikon Eclipse Tsi2 (Tokyo, Japan).

Proliferation Assay
pDASMC proliferation was evaluated using a BrdU colorimetric cell proliferation ELISA kit (Roche). pDASMCs were plated into a 96-well culture plate with DMEM containing 10% FBS at an initial density of 5x10^3 cells/well and cultured for 48h. After 24-h serum starvation, cells were stimulated with or without BMZ (20, 50, or 100 µmol/L). Administration of 10% FBS was used for positive controls. Cells were then incubated with BrdU (10 µmol/L) for 24h. Incorporation of BrdU was quantified according to the manufacturer’s instructions.

In Vivo BMZ Administration and Analyses of Lung Maturation and IT Formation
Pregnant rats were injected intravenously with saline or BMZ (0.4 mg/kg) on gestational days 18 and 19, and were euthanized by injection of pentobarbital (200 mg/kg) on gestational day 20. We determined the dose of BMZ in reference to the previous study that demonstrated BMZ-induced effects in the lung in vivo. After euthanization, fetal arteries, including the DA and the aortic arch, and the lungs were removed from the thoracic cavity and fixed in buffered 10% formalin, and embedded in paraffin. The sectional segments in the middle portion of the DA were used for Elasticia van Gieson staining. Lung morphometry was performed as previously described. We analyzed lung maturation with airspace volume density. Airspace volume density was calculated by dividing the sum of the airspace area by the total area. IT formation was defined as neo-intima area/media area using Image J software, as described previously.

Statistical Analysis
All values are shown as the mean±standard error of the mean (SEM) of at least three independent experiments. A 2-way ANOVA followed by Bonferroni correction was used to analyze the data of Figures 1A, 1B, 2A, and 2C. The data of Figures 1C, 2A and 2D were analyzed by the Kruskal-Wallis test, followed by Fisher’s least significant difference post hoc test. The data shown in Figures 3D, 4B and 4D were statistically analyzed by Mann-Whitney U test. A value of P<0.05 was considered statistically significant.

Results
Microarray Data Revealed BMZ-Induced Genes in Rat pDASMCs
To investigate the effect of BMZ on gene expression in SMCs isolated from preterm infants, we performed DNA
microarray analysis of rat pDASMCs stimulated with or without BMZ. Among a total of 58,717 probe sets, 20 genes showed upregulation (>3.0-fold) after stimulation by BMZ, and ADP-ribosyltransferase 3 (Ar3) was the most upregulated gene, although expression of 18S ribosomal RNA (18S) was not changed (Table). We also observed that known glucocorticoid-regulating genes, such as Fk506-binding protein 5 (FKBP5), 38 glucocorticoid-inducible kinase 1 (SGK1), 34 and Tsc22d3 37 were upregulated by 1.7-, 1.3-, and 2.0-fold, respectively, in pDASMCs.

ADP ribosyltransferases (Arts) alter cell functions by catalyzing mono-ADP-ribosylation, which is a post-translational protein modification. 38 It has been reported that Ar3 promotes cell migration and proliferation in melanoma cells, 23,39 which are important cellular processes for IT formation. We, therefore, focused on the roles of Ar3 in SMC migration.

**BMZ Increased Ar3 mRNA in Rat DASMCs**

We then quantified Ar3 mRNA using real-time RT-PCR in rat pDASMCs and pASMCs stimulated with or without BMZ. The expression level of Ar3 was markedly increased in BMZ-treated pDASMCs, but not in pASMCs, in a dose-dependent manner (Figure 1A). In accordance with the microarray data, expression levels of 18S were statistically similar between control and BMZ-treated groups in pDASMCs (data not shown). BMZ-increased Ar3 mRNA was observed in a time-dependent manner, which was suppressed by actinomycin D (Figure 1B).

Glucocorticoid binds both to glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). 40 A previous report demonstrated that GR, but not MR, was responsible for glucocorticoid-induced Ar3 expression. 41 We observed that GR (Ntr3c1) was present in both pDASMCs and pASMCs, and the expression level of GR (Ntr3c1) was similar between these 2 cell types, which were not affected by BMZ stimulation (Figure 1C).

**BMZ Promoted DASMC Migration But Not Proliferation**

To investigate whether BMZ promotes the migration of SMCs of preterm DA via Ar3 upregulation, we performed a scratch assay using rat pDASMCs and 2 types of Ar3-targeted siRNAs. First, we confirmed that both Ar3-targeted siRNAs significantly decreased Ar3 expression (Figure 2A). We found that BMZ stimulation significantly promoted migration of pDASMCs transfected with negative siRNA (control) in a time-dependent manner (Figure 2B and 2C, red vs. black lines). This effect was attenuated in pDASMCs transfected with Ar3-targeted siRNA (Figure 2B and 2C, red vs. blue lines).

When we stimulated pDASMCs with 10% FBS, cell proliferation was significantly increased (Figure 2D). However, BMZ did not induce pDASMC proliferation detected by changes in BrdU incorporation (Figure 2D). These findings suggest that BMZ stimulation promoted pDASMC migration via enhancement of Ar3 expression, and BMZ was not related to SMC proliferation in the DA.

**Effect of BMZ on SMC Morphology**

We further examined migration-related morphological changes in pDASMCs and found that BMZ stimulation increased the percentage of BMZ-induced lamellipodia formation (Figure 3A, B), which supports the findings of BMZ-induced migration. It has been reported that paxillin was involved in glucocorticoid-induced migration. 44 Immunocytochemistry demonstrated that phosphorylated paxillin seemed to be increased in pDASMCs treated with BMZ (Figure 3A). We then quantified changes of paxillin protein expression in whole cell lysates. BMZ significantly increased total paxillin expression and the ratio of phosphorylated to total paxillin (Figure 3C, D).

**Antenatal BMZ Promoted IT Formation in Preterm Rat DA**

Finally, we examined whether antenatal BMZ administration promotes IT formation in preterm rat fetuses in vivo. Pregnant rats were administered BMZ on gestational days 18 and 19, and fetuses were analyzed on gestational day 20. The period between gestational days 18–20 in rats is equivalent to the second trimester pregnancy in humans. 42 Antenatal BMZ administration increased airspace volume density (Figure 4A, 4B), suggesting that this treatment promoted lung maturation as reported previously. 42 Elastica van Gieson staining revealed that antenatal BMZ administration significantly promoted IT formation in preterm rat fetuses (Figure 4C, 4D).

**Discussion**

In the present study, we demonstrated that BMZ promoted the migration of SMCs isolated from rat preterm DA via Ar3 and enhanced IT formation in preterm rat DA. To the best of our knowledge, this is the first report showing glucocorticoid-mediated DA remodeling; that is, IT formation.

We performed transcriptional profiling of rat pDASMCs treated with BMZ, and identified Ar3 as a BMZ-induced transcript in the preterm rat DA, although the protein expression of Ar3 could not be examined due to a lack of specificity in commercially available Ar3 antibodies. Mammalian ecto ADP-ribosyltransferases (ArTs) catalyze mono-ADP-ribosylation, which is a posttranslational protein modification, and regulate the biological functions of various types of cells via the transfer of a single ADP-ribosyl moiety from NAD+ to a specific amino acid in a target protein. 45 Of five human ArTs (ART1–ART5), ART3 was cloned from human testis. 46 The expression of ART3 was demonstrated in human heart, skeletal muscles, small intestine, breast cancer cells, and melanoma cells. 47–49 Recently, Shenel et al reported the effects of antenatal BMZ exposure on gene expression in mouse and baboon DAs. 51 The authors focused on DA contraction and found in both mouse and baboon DAs that BMZ increased the expression of potassium channels and phosphodiesterases, which mediate vascular constriction. However, the report did not refer to an expression change of ART3. In other cell types, it has been reported that ART3 expression was increased in mouse cardiomyocytes treated with corticosterone 44 and rat neurons in the brain cortex after traumatic brain injury. 46 Muller et al reported that using GR (null/null) or MR (null/null) mice revealed that GR, but not MR, was responsible for glucocorticoid-induced ART3 expression. 46 Our data demonstrated that BMZ increased Ar3 in pDASMCs, which was not observed in pASMCs, while GR expression was similar between pDASMCs and pASMCs. These data suggest that DA-specific intracellular signaling pathways for Ar3 transcriptional regulation may exist.

It has been reported that the proximal promoter of the forkhead box O1 (FOXO1) gene contains multiple functional glucocorticoid response elements (GREs) and that
FOXO1 is known as a glucocorticoid-induced gene.\(^{47}\) *Ar1* has the transcription factor binding site FOXO1 in the regions spanning up to 4 kb around its transcription starting sites. We then examined whether FOXO1 was associated with BMZ-induced *Ar1* expression, but the data did not show the involvement of FOXO1 in *Ar1* expression (data not shown). To date, no report indicating the molecular mechanisms of transcriptional regulation of *Ar1* has been published. Further investigation is needed to elucidate this mechanism.

In the present study, we demonstrated BMZ-mediated pDASMC migration. To date, only one investigation has indicated the role of glucocorticoid in vascular SMC migration by showing that dexamethasone did not affect migration in cells isolated from lamb DA.\(^{44}\) In other cell types, such as mesenchymal stem cells and melanoma cells, however, it has been demonstrated that glucocorticoid promotes migration.\(^{28-27}\) Yun et al showed that dexamethasone stimulated human mesenchymal stem cell migration through the expression of FAK and paxillin,\(^{36}\) both of which are essential for migration.\(^{48,49}\) In the present study, total and phosphorylated paxillin expressions were increased by BMZ, but BMZ did not have an effect on protein expression of focal adhesion kinase (FAK) (data not shown). These data suggested that glucocorticoid-induced migration-related intracellular signaling pathways were partially shared between multiple cell types.

Unlike other ART subtypes, ART3 does not have arginine-specific enzyme activity due to a lack of the active site motif (R-S-EXE) that is essential for catalytic activity,\(^{51}\) and the biological function of ART3 remains largely unknown. In the present study, BMZ-mediated migration was attenuated in pDASMCs transfected with *Ar1*-targeted siRNA. A few studies using ART3 silencing suggested that ART3 promoted migration/invasion in breast cancer cells\(^{43}\) and melanoma cells.\(^{44}\) It seems likely that ART3 promotes migration in several cell types. Phosphorylation of paxillin occurred in parallel with migration of pDASMCs in our study. More work will be required to elucidate the direct target of ART3 that regulates paxillin activation and/or cell migration.

We administrated 2 doses of BMZ at a 24-h interval in pregnant rats and found that antenatal BMZ moderately, but significantly, promoted IT formation in the DA. It has been demonstrated that the contractile effect of glucocorticoids on the DA was reversible and began to wane several hours after glucocorticoid administration.\(^{40,41}\) BMZ-induced IT formation may also be time-course dependent. The regimens of antenatal glucocorticoid administration to the mother prior to anticipated preterm birth have been extensively examined. Based on these studies investigating various dosages of drugs\(^{45,49}\) and the time-course of administration,\(^{45,49}\) most women currently receive a single-course administration of 2 doses of 12 mg BMZ at a 24-h interval for RDS prevention.\(^{46}\) Shelton et al first demonstrated an association of the time-course of antenatal BMZ administration and DA closure in humans.\(^{14}\) In this study, the infants were divided into 2 groups depending on the interval between the first dose of antenatal BMZ and delivery. The infants who were delivered during the interval between 7 h and 9 days following the first BMZ dose had reduced the incidence of moderate-to-large PDA, compared to the infants who received a different time-course of BMZ administration.\(^{10}\) Further study on the effect of the antenatal glucocorticoid administration regimen on DA closure in humans would have the potential to improve hemodynamically significant PDA prevalence.

In conclusion, antenatal BMZ administration may contribute to IT formation in the DA through *Ar1*-mediated DA-SMC migration.\(^{14}\) Together with the findings of previous studies focusing on DA contraction,\(^{13-15}\) our data suggest the beneficial role of antenatal BMZ in DA closure.

**Disclosures**

None.

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**Author Contributions**

T.K. and U.Y. conceived and designed the experiments. T.K., J.S., and Satoko Ito performed the experiments. T.K. and U.Y. analyzed the data. A.U., S.N., Shiko Iwaisaki, K.S., and Shuichi Ito aided in experimental design. T.K., U.Y., and Y.I. wrote the paper.

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II 副論文

該当無し

III 参考論文

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