Antenatal Administration of Betamethasone Contributes to Intimal thickening of the Ductus Arteriosus

Takahiro Kemmotsu[1)2)], Utako Yokoyama[2)], Seiko Azuma[2)], Junichi Saito[2)], Satoko Ito[2)], Azusa Uozumi[1)], Shiho Iwasaki[3)], Shigeru Nishimaki[1)], Shuichi Ito[1)], Munetaka Masuda[4)], Toshihide Asou[5)], Yoshihiro Ishikawa[2)]

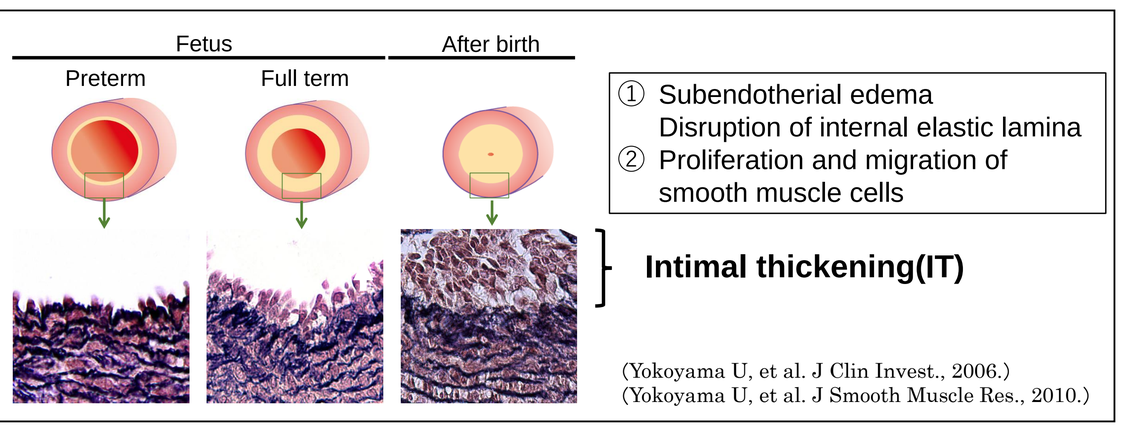
1) Department of Pediatrics, Yokohama City University
2) Cardiovascular Research Institute, Yokohama City University
3) Perinatal Center, Yokohama City University Medical Center
4) Department of Cardiovascular Surgery, Yokohama City University
5) Department of Cardiovascular Surgery, Kanagawa Children's Medical Center

BACKGROUNDS

- Antenatal betamethasone (BTM) administration is widely accepted to reduce respiratory distress syndrome. In addition, some observational studies indicate that BTM decreases prevalence of patent ductus arteriosus (PDA) in preterm infants.

Author	Study period	Inclusion criteria	PDA reduction rate
Morales et al.	1986-1988	Gestational age 26-34w (n=165)	67%
Amorim et al.	1997-1998	Gestational age 26-34w (n=218)	73%
Elimian et al.	1990-1997	Birth weight 500-1750g (n=527)	44%
Been et al.	2001-2003	Gestational age <32w (n=121)	44%

- Closure of the ductus arteriosus (DA) requires morphological remodeling, i.e., intimal thickening (IT) formation. However, the role of BTM in IT formation of the preterm DA has not been reported.



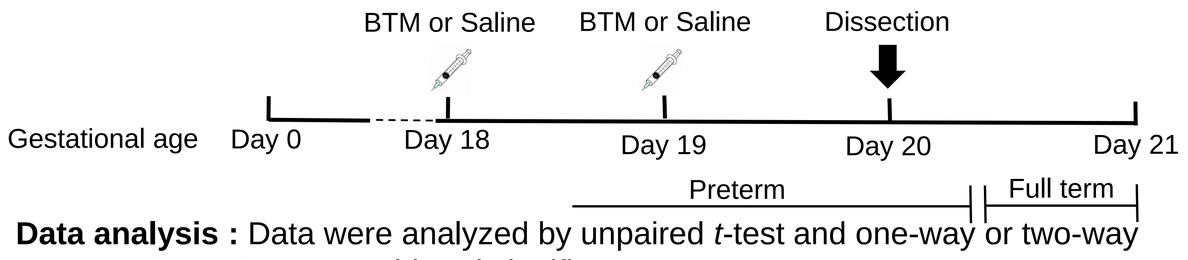
OBJECTIVE

To examine the role of antenatal BTM in DA IT formation.

MATERIALS AND METHODS

- **Tissues :** Wistar fetal rats were obtained from time-pregnant mother (SLC, Japan).
- **Cells :** Smooth muscle cells of rat DA (DASMCs) and smooth muscle cells of rat aorta (ASMCs) on day 20 of gestation were obtained by primary culture.
- **Reagents :** Anti-glucocorticoid receptor antibodies (Cell Signaling technology, USA) and betamethasone sodium phosphate (WAKO, Japan) were used. A BrdU Cell Proliferation Assay Kit (Sigma-Aldrich, USA) was used.
- **Expression of mRNAs :** qRT-PCR using SYBR Green was performed.
- **Microarray analysis :** SurePrint G3 Rat GE v2 8x60K Microarray (Agilent, Japan).
- **Scratch assay :** Half of DASMCs on cell culture dish were shaved off and migration area was measured at 24-72 h after BTM stimulation.

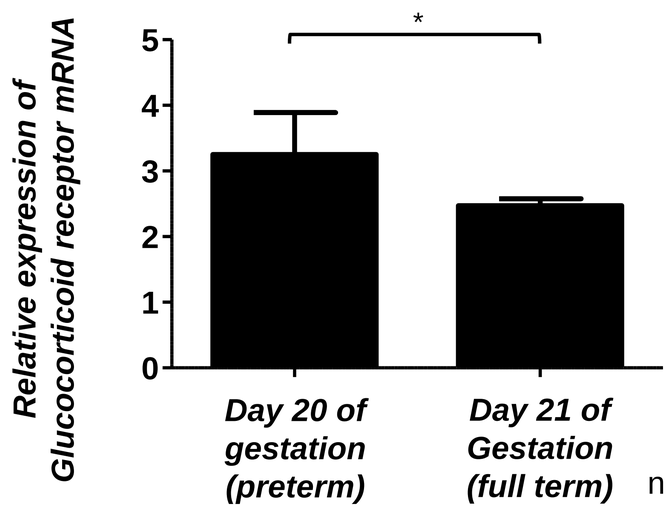
- **Antenatal BTM administration :** Maternal rats were administered intravenously with BTM 0.4mg/kg or normal saline (control) and fetal rat DA tissues were obtained. After elastica van Gieson (EV) staining, IT area were measured by image J software.



- **Data analysis :** Data were analyzed by unpaired *t*-test and one-way or two-way ANOVA. *P* <0.05 was considered significant.

RESULTS

Figure 1. Glucocorticoid receptor was expressed in preterm rat DA tissues.



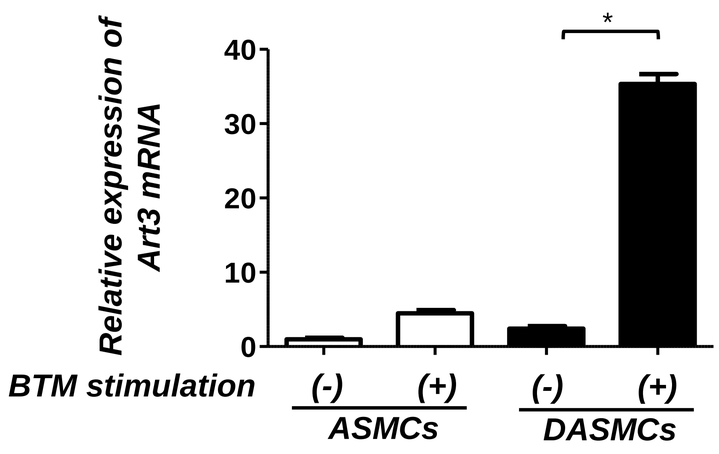
n = 5, * *P* <0.05 (unpaired *t*-test)

Figure 2. Microarray data revealed BTM-induced genes in rat DASMCs

DASMCs(day 20 of gestation) were stimulated by BTM 100nmol/ml
↓
58,717genes were detected
↓
109 probe sets were > 2.0-fold increased by BTM stimulation. (*P* <0.05)

Gene Symbol	mRNA description	Fold change (BTM/Control)
Art3	ADP-ribosyltransferase 3	11.1
Scgb2a2	Secretoglobin, family 2A, member 2	8.40
Rapgef5	Rap guanine nucleotide exchange factor 5	4.06
Xdh	Xanthine dehydrogenase	2.50
Lpar1	Lysophosphatidic acid receptor 1	2.38
⋮	⋮	⋮

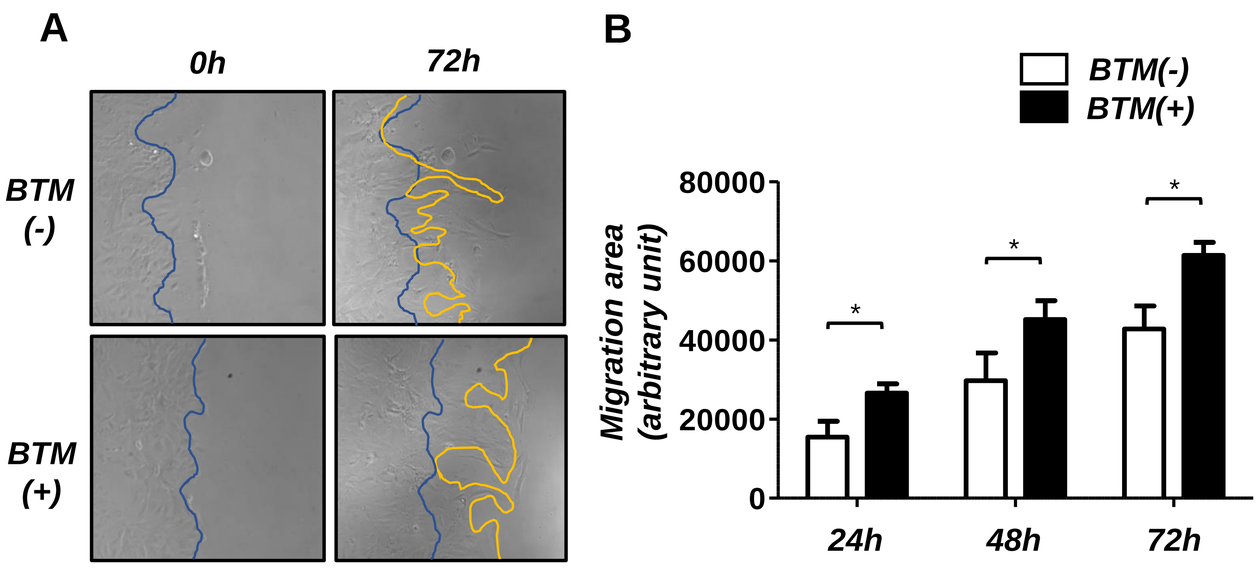
Figure 3. BTM increased Art3 mRNA in rat DASMCs.



n = 5, * *P* <0.05 (unpaired t-test)

RESULTS

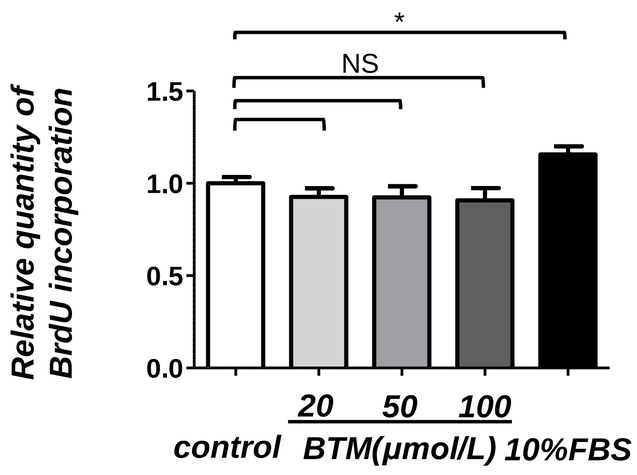
Figure 4. BTM promoted DASMC migration.



Blue line and yellow line indicate cell area at the start and 72 h after stimulation, respectively.

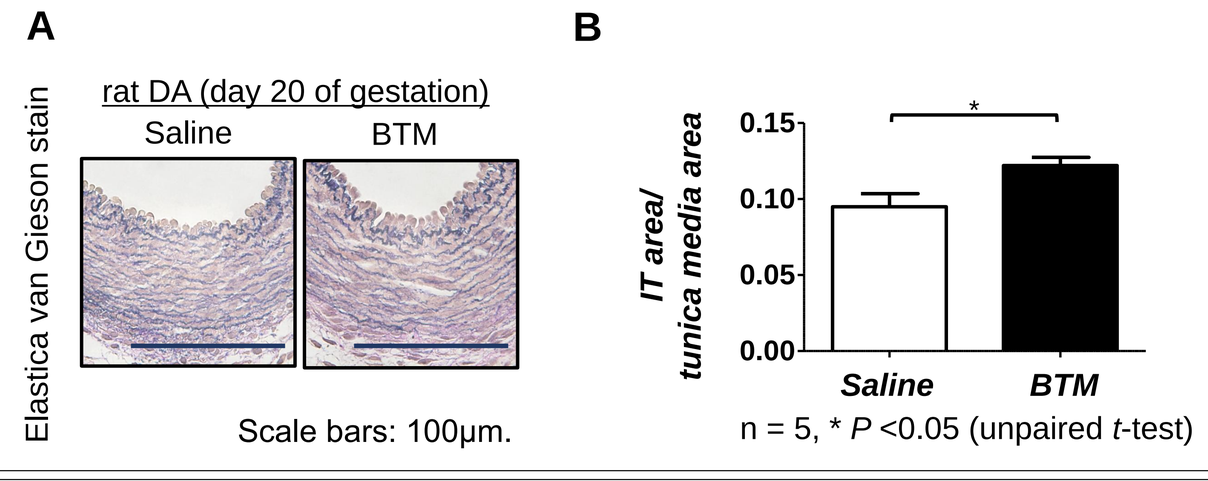
n = 5, * *P* <0.05 (two-way ANOVA)

Figure 5. BTM did not promote DASMC proliferation.



n = 4, NS; not significant, * *P* <0.05 (one-way ANOVA)

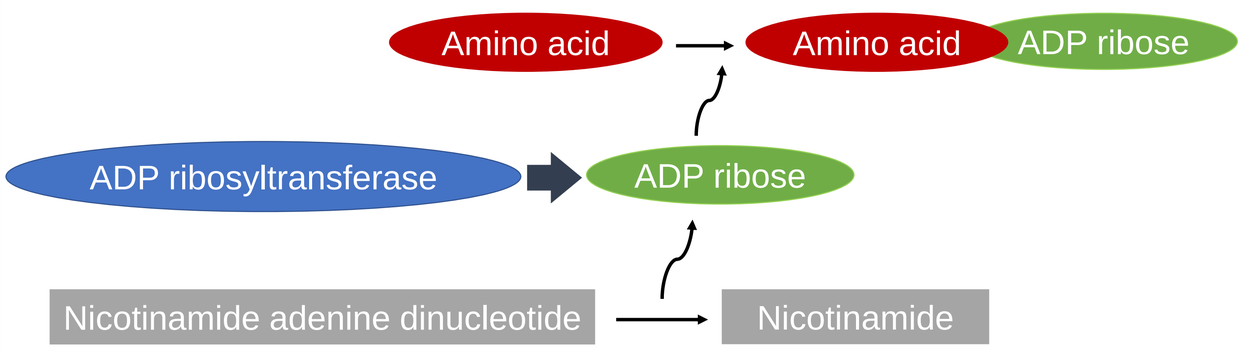
Figure 6. Antenatal BTM promoted IT formation in preterm rat DA.



n = 5, * *P* <0.05 (unpaired *t*-test)

About Art3 (ADP-ribosyltransferase3)

- Art3 catalyzes ADP ribosylation.



(Levy I, et al. FEBS Lett., 1996)
(Okazaki I, et al. Annu Rev Nutr., 1999)

- ADP ribosylation is associated with smooth muscle cell migration via actin regulation.

(Terashima M. Eur J Biochem., 1992)
(Saxty B. Biochem Soc Trans., 1998)

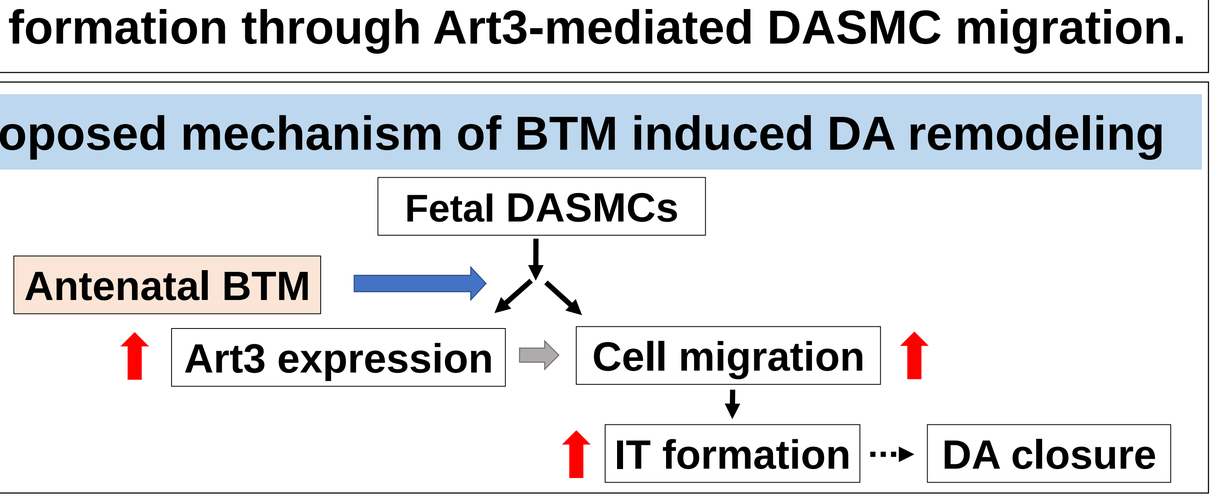
- Art3 expression is enhanced by glucocorticoid in rat cardiomyocytes.

(Olivier M. Genomics., 2007)

CONCLUSIONS

Antenatal BTM administration may contribute to DA IT formation through Art3-mediated DASMC migration.

Proposed mechanism of BTM induced DA remodeling



The authors have no financial conflicts of interest to disclose concerning the presentation.