unlikely to produce an unfavorable effect on respiration when used as DA dilators. It should be noted that PDE3 inhibitors have adverse effects, such as arrhythmia or hypotension [33]. Milrinone reduces the risk of low cardiac output syndrome for some pediatric patients after congenital heart surgery; however, milrinone use is an independent risk factor for clinically significant tachyarrhythmias [34]. Although it was not feasible to examine arrhythmias and change in blood pressure in rat neonates in this study, careful further study is warranted to examine adverse effects.

It should be emphasized that both the PDE3a protein and the PDE3b protein were abundantly detected in the smooth muscle layer and the IT layer in all human DA samples tested, regardless of the patient's diagnosis or age at the time of operation (Figure 7). A previous study demonstrated that PDE3 inhibitors prevented DA closure in premature infants with persistent pulmonary hypertension [15, 35, 36]. Together with these findings, those of the present study suggest that PDE3 inhibitors can dilate the DA without inducing intimal thickening, and that they may serve as alternatives to PGE_1, the current DA vasodilator used for patients with DA-dependent CHDs.

Acknowledgments

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Figure Legends

Figure 1. Quantitative RT-PCR analyses of PDE3a, PDE3b, and EP4 in rat e21 DA, aorta, and pulmonary artery (PA) tissue. n = 4–5, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, NS indicates not significant.

Figure 2. The effects of milrinone and olprinone on vasodilation of the DA as observed by the rapid whole-body freezing method. (A) PGE_1 (10 μg/kg)-induced dilation of rat DA (n = 4–6). (B) Vasodilatory effect of milrinone on rat DA. Rat neonates were intraperitoneally injected with milrinone (n = 4–6). (C) Representative images of rat DAs treated with 10 mg/kg of milrinone or saline (control) for 2 h using the whole-body

freezing method (arrow). (D) Vasodilatory effect of milrinone on rat DA. Rat neonates were intraperitoneally injected with olprinone (n = 4–6). (E) Representative images of rat DAs treated with 5 mg/kg of olprinone or control for 2 h using the whole-body freezing method (arrow). (F) Milrinone or olprinone dilated DA in a dose-dependent manner. Vasodilatory effects of PDE3 inhibitors were examined 2 h after injection (n = 4–6). *** $p < 0.001$ and NS vs. control. NS indicates not significant.

Figure 3. Effects of PDE3 inhibitors and PGE_1 on respiratory distress. (A) Respiratory rate of rat neonates administered each drug immediately after birth, the same as in Figure 2 (n = 6–9). (B) Respiratory rate of rat neonates administered each drug 2 h after birth (n = 4). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. control. No mark indicates not significant vs. control.

Figure 4. Milrinone increased cAMP production, however, it did not induce HA production. (A) Milrinone (10 μM) significantly increased cAMP accumulation in DASMCs (n = 4). (B) HA production in SMCs treated with milrinone (10 μM), cilostazol (10 μM), rolipram (10 μM), PGE_1 (1 μM), or PGE_2 (1 μM) (n = 4–6). Cilostazol: PDE3 inhibitor. Rolipram: PDE4 inhibitor. **$p < 0.01$ and ***$p < 0.001$ vs. control. No mark

indicates not significant vs. control.

Figure 5. Milrinone did not promote migration and proliferation in SMCs. (A) Migration of SMCs treated with milrinone (10 μM), PGE_1 (1 μM), or PDGF-BB (10 ng/ml) using the Boyden chamber method (n = 4–5). (B) Proliferation of SMCs treated with milrinone (10 μM) or PGE_1 (1 μM) in the presence of 0 or 10% FBS by an MTT assay (n = 5–9). $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. NS indicates not significant.

Figure 6. Effect of co-treatment of HA with milrinone on migration and proliferation in DASMCs. (A) Migration of SMCs with co-treatment of HA (200 ng/ml) and milrinone (10 μM) using the Boyden chamber method (n = 4–5). (B) Proliferation of SMCs with co-treatment of HA (200 ng/ml) and milrinone (10 μM) in the presence of 0 or 10% FBS by an MTT assay (n = 8). $**p < 0.01$, NS indicates not significant.

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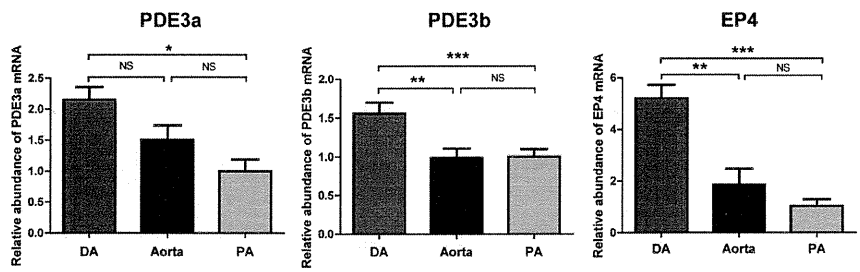
Fig. 1



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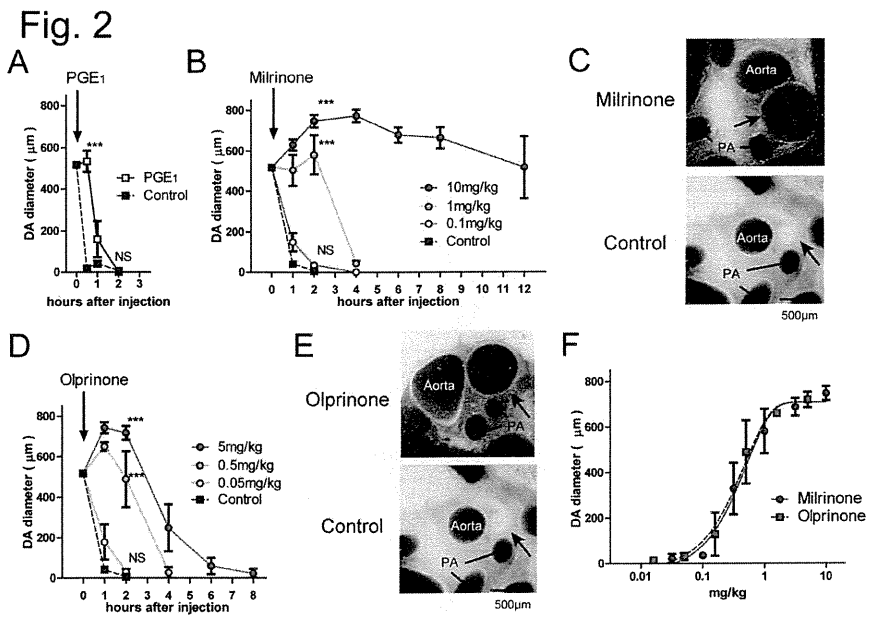

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Fig. 3

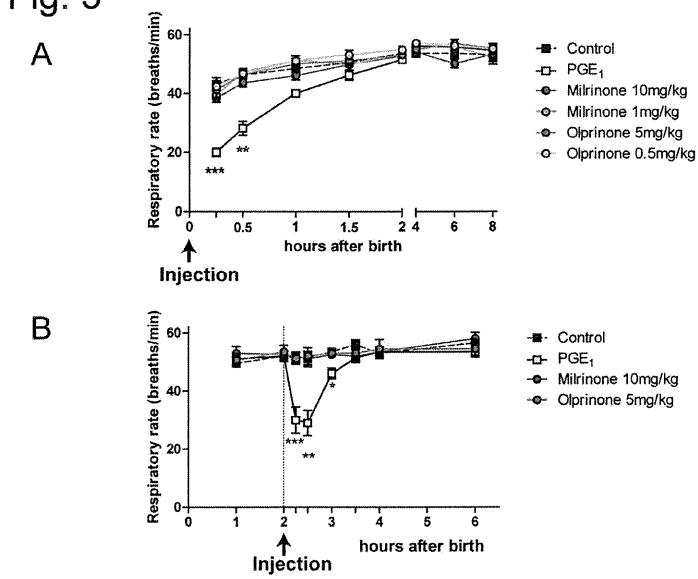

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Fig. 4

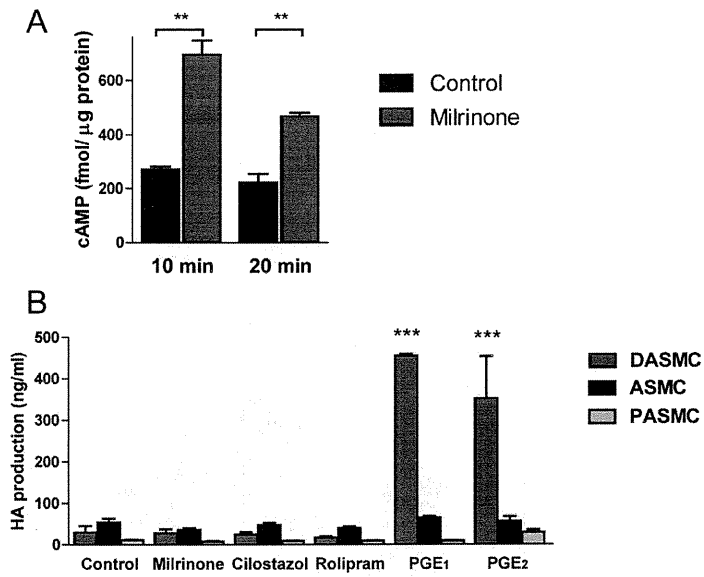

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Fig. 5

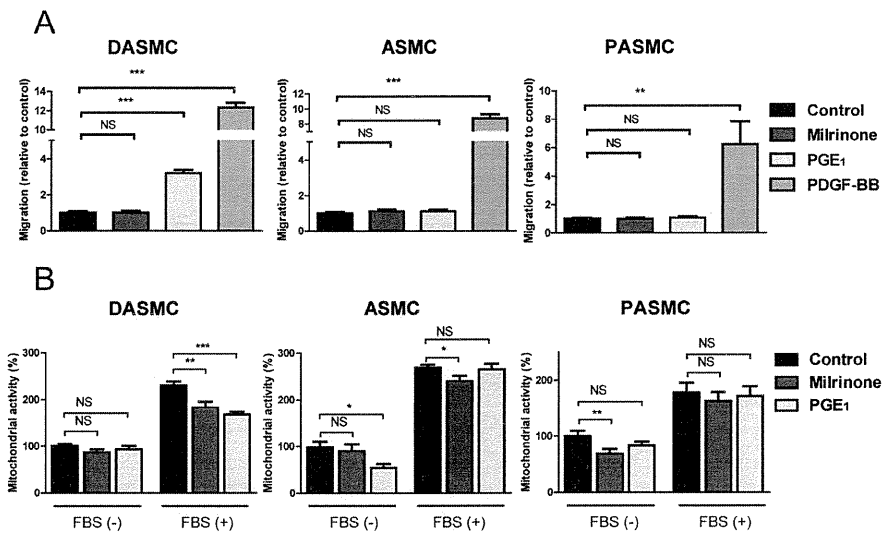

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Fig. 6

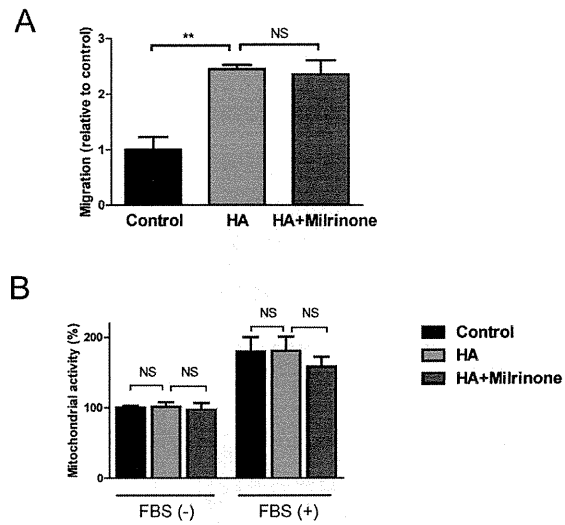

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Fig. 7

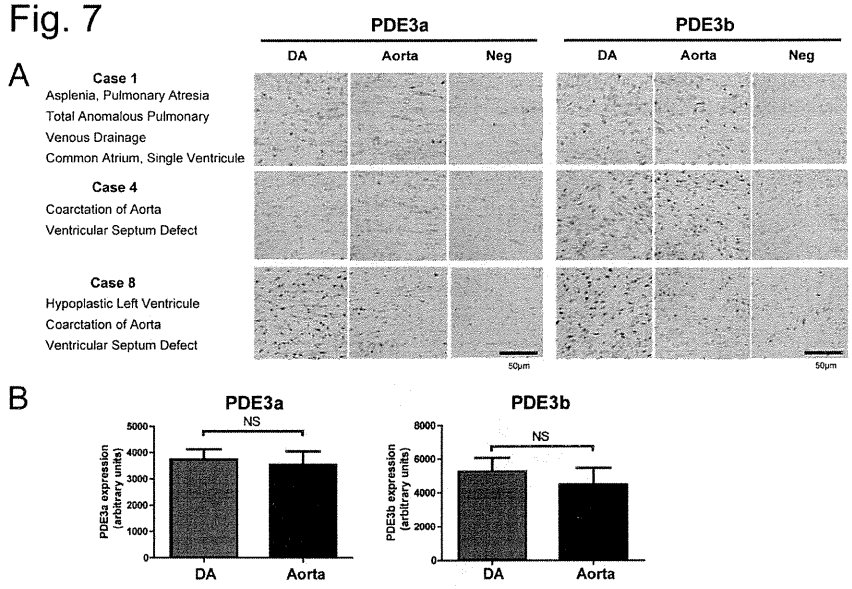

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Table 1.

Summary of patient characteristics

Case No.	Age at Operation	Diagnosis
1	0 days	Asplenia, PA, TAPVD, CA, SV
2	1 day	Asplenia, CoA, CA, SV
3	2 days	IAA, Aorticopulmonary window
4	2 days	CoA, VSD
5	3 days	TGA, CoA
6	4 days	CoA, VSD
7	13 days	CoA, VSD
8	1 month	hypoLV, CoA, VSD

PA: Pulmonary Atresia, TAPVD: Total Anomalous Pulmonary Venous Drainage,
CA: Common Atrium, SV: Single Ventricule,
CoA: Coarctation of Aorta, IAA: Interruption of Aortic Arch,
VSD: Ventricular Septum Defect, TGA: Transposition of the Great Arteries,
hypoLV: Hypoplastic Left Ventricule