Acta Med. Nagasaki 50: 29－33

Phosphodiesterase V Inhibitors Dilate the Pulmonary Artery of Monocrotaline-Induced Pulmonary Hypertensive Rats

Tetsuji MAKITA,[1] Yohsuke TSUTSUMI,[2] Sungsam CHO,[1] Osamu SHIBATA,[2] Koji SUMIKAWA[2]

[1] Division of Intensive Care Unit, Nagasaki University Hospital, Nagasaki, Japan

[2] Division of Anesthesiology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Treatment of pulmonary hypertension has not yet been established and effective drug therapies are required. We hypothesized that inhibition of cyclic guanosine monophoshate (cGMP)- phosphodiesterase (PDE) would result in specific vasodilation of the hypertensive pulmonary arteries. This study was carried out to determine whether PDE V inhibitors could dilate pulmonary artery (PA) from monocrotaline-induced pulmonary hypertensive rats. Thirty-six Wistar rats were given either monocrotaline (105 mg/kg) or normal saline (control) subcutaneously. Three weeks later, the PA rings were isolated and mounted in 5-mL organ chambers. After precontraction with norepinephrine (0.1 μmM), one of two PDE V inhibitors, zaprinast and dipyridamole, was added in a cumulative fashion. We also investigated whether nitric oxide synthetase (NOS) inhibitor modifies the effects of the PDE V inhibitors on the PA. Zaprinast and dipyridamole dose-dependently dilated the PA from either saline- or monocrotaline- treated rats. There was no difference in the dilatory effect between monocrotaline and saline rats. Pretreatment of a NOS inhibitor (N^G-nitro-L-arginine methyl ester (0.1 mM)) reduced moderately the vasodilatory effect of zaprinast on the PA from monocrotaline-treated but not saline-treated rats. The results suggest that PDE V inhibitors exert a strong vasodilating effect on PA of pulmonary hypertensive rats as well as on that of normal rats, and that NO plays a role, at least in part, in the effect of PDE V inhibition on PA of pulmonary hypertensive rats.

ACTA MEDICA NAGASAKIENSIA 50: 29－33, 2005

Keywords: PDE V inhibitor; Monocrotaline; Rat; Pulmonary hypertension

Introduction

Primary pulmonary hypertension is uncommon but has poor prognosis. Secondary pulmonary hypertension is relatively common, and leads to a significant reduction in life expectancy.[1] The treatment of pulmonary hypertension has not yet been established and effective drug therapies are needed.

It is known that the essential therapy for pulmonary hypertension is to selectively dilate the pulmonary artery. One of the vasodilating mechanism is the accumulation of cyclic 3'-5'-guanosine monophosphate (cGMP), which inhibits calcium release from the sacroplasmic reticlum in the smooth muscle cell.

There are at least seven isoforms of mammalian phosphodiesterase (PDE) with varying specificity for hydrolysis of cyclic nucleotides.[2] Human pulmonary arteries contain four PDE isozyme families (I, III, IV, V) as identified by Fast protein liquid chromatography (FPLC) and isozyme inhibition by specific inhibitors.[3] It is known that the inhibitors of PDE III, IV and V relax isolated normal human pulmonary arteries.[3] PDE V hydrolyzes cGMP specifically,[4,5] and thus PDE V inhibitors increase intracellular cGMP, which subsequently decreases cytoplasmic Ca^{2+} concentration and relaxes vascular smooth muscle. Despite increased endogenous nitric oxide (NO) and cGMP production in the pulmonary hypertensive circulation, additional vasodilation can be produced through further cGMP accumulation in response to PDE V inhibition.[2]

Recently PDE V inhibitors are used for the patients with erectile dysfunction because of their selective penile vasodilating effects.[6,7] On the other hand, it was reported that a PDE V inhibitor markedly attenuated the rebound pulmonary hypertension caused by withdrawal of inhaled NO.[8] We therefore would like to know that the administration of PDE V inhibitors alone could directly dilate pulmonary hypertensive artery without co-administration of NO.

We hypothesized that inhibition of cGMP-PDE would result in specific vasodilation of the hypertensive pulmonary arteries. It has

Address correspondence: Tetsuji Makita, M.D., Division of Intensive Care Unit, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki 852-8501 JAPAN

TEL: +81-(0)95-849-7370, FAX: +81-(0)95-849-7379, E-mail: makita@net.nagasaki-u.ac.jp

Received October 28, 2004; Accepted February 12, 2005

been known that the administration of monocrotaline to rats leads to the development of pulmonary hypertension and right ventricular hypertrophy. Therefore we carried out this study to determine whether PDE V inhibitors, zaprinast and dipyridamole, could dilate pulmonary artery (PA) from monocrotaline-induced pulmonary hypertensive rats. We also investigated the influence of a NOS inhibitor on the effects of PDE V inhibitors to determine the role of NO on the pulmonary vasodilating effects of zaprinast and dipyridamole.

Methods

The protocol of this study was approved by the Nagasaki University Institutional Animal Care Committee.

Drugs

The following drugs were used: monocrotaline, norepinephrine, acetylcholine, zaprinast, dipyridamole, and N^G-nitro-L-arginine methyl ester (Sigma Chemical, St. Louis, MO). The concentration of the drugs were expressed as final molar concentration in the bath solution. The stock solution of zaprinast was dissolved in NaOH (0.05N) and then diluted in distilled water. The stock solution of dipyridamole was dissolved in ethanol and then diluted in distilled water. Monocrotaline was dissolved in HCl (1.0N) and pH was adjusted to 7.4 with NaOH (1.0N). The other substances were prepared in distilled water. The final concentration of the solvents in the organ bath was less than 1.0%.

Monocrotaline-induced pulmonary hypertensive rats

Wistar rats weighing 200-250 g were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg), and then given monocrotaline (105 mg/kg) or saline (1 mL/kg) subcutaneously 3 weeks prior to the experiments.[9] To determine the extent of monocrotaline-induced right ventricular hypertrophy, the ventricular free wall (RV) was cut from the left ventricle and septum (LV+S) and weighed separately. The ventricular weight ratio was determined from RV/(LV+S).

Tissue preparation

Three weeks later, 19 rats given monocrotaline and 17 rats given saline were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). The chest cavity was opened and 100 IU of heparin was injected into the right ventricle. After the rats were exsanguinated, the heart and lungs were removed. The trunk and extrahilar PA were carefully dissected and cut into two or three rings of 2-3 mm width. Care was taken during harvesting the blood vessels not to touch the inner surface of the blood vessels.

Organ bath technique

The rings were mounted horizontally in 5-mL Krebs-Henseleit solution (composition in mmol/L: NaCl, 118; KCl, 4.69; $CaCl_2$, 3.35; $MgSO_4$, 1.18; KH_2PO_4, 1.04; $NaHCO_3$, 25; D-Glucose, 11.1; pH, 7.40±0.05) aerated with 95% O_2-5% CO_2 at 37±0.5 ℃. The one side hook was connected to a force transducer (UFER, Kyoto, Japan), and changes in isometric force were recorded and stored for later analysis on a Macintosh (4400/200) computer using the MacLab 8 data collection system (AD Instruments Inc., Milford, MA).

The resting tensions (750 mg) defined by preliminary studies were progressively applied, and the rings were allowed to equilibrate for 30 minutes. The rings were exposed to 80 mM of KCl to confirm the integrity of smooth muscle cells. The rings which did not constrict up to 750 mg were discarded. The remaining rings were then washed with Krebs-Henseleit solution three times followed by a second equilibration period of 30 minutes at the minimum. After the second equilibration period, the rings were constricted with norepinephrine (NE) of 0.1 μM at a final concentration which caused a submaximal contraction (75-80 % of maximal constriction induced by NE) in a preliminary study. Fifteen minutes later, acetylcholine of 1 μM was added to confirm that muscarinic receptors were functioning on the endothelium. The rings which did not dilate with acetylcholine were discarded. Then the remaining rings were washed with Krebs-Henseleit solution several times followed by a third equilibration period of 90 minutes at the minimum.[10]

After the third equilibration period, NE of 0.1 μM was added to induce a submaximal contraction and 15 minutes were allowed to reach equilibrium. Thereafter, zaprinast of 10 nM to 0.1 mM at final concentrations or dipyridamole of 10 nM to 0.1 mM at final concentrations was added to the bath in a cumulative fashion with at least 5 minutes' interval, in which the relaxation reached plateau. Each PDE V inhibitors has been studied from the other rats. The effects of vehicle were also checked on the contraction and dilation responses in three of the saline- or monocrotaline-treated rats, respectively.

Influences of NOS inhibitor

After the third equilibration period, N^G-nitro-L-arginine methyl ester (L-NAME 0.1 μM) was added on PA rings from either saline- or monocrotaline-treated group. Fifteen minutes later, NE of 0.1 mM was added to induce an optimal contraction followed by an equilibration period of 15 minutes. Thereafter, zaprinast of 10 nM to 0.1 mM or dipyridamole of 10 nM to 0.1 mM was added to the bath in a cumulative fashion.

Data and statistical analysis

In all experiments, n represented the number of rats from which the vessel rings were obtained. The relaxation caused by PDE V inhibitors was expressed as percent decrease in the tension of the vessel rings preconstricted by NE. Data were summarized as mean

±standard deviation (SD). Dose-dependency of dilatation of PA by zaprinast or dipyridamole was analyzed by repeated measures analysis of variance. When a significant dose-dependency was observed, significance of dilatation of PA at each dose was tested against baseline by t-test for zaprinast and dipyridamole; the significance level of each test was set at 0.0056 by Bonferroni inequality to keep the overall significance level at 0.05. Influence of NOS inhibitor was examined by comparing dilation of PA between the two groups with and without L-NAME by t-test. MEANS and GLM in the SAS® system[11] were used for the calculations.

Results

The weight ratio of RV/(LV+S) were significantly (p<0.0001) greater in monocrotaline-treated group (0.41±0.1, n=19) compared to saline-treated group (0.25±0.02, n=17).

Figure 1 shows a typical recording of the dilatory effect of PDE V inhibitors on PA from monocrotaline-induced pulmonary hypertensive rats. Vehicle of PDE V inhibitors produced no dilation.

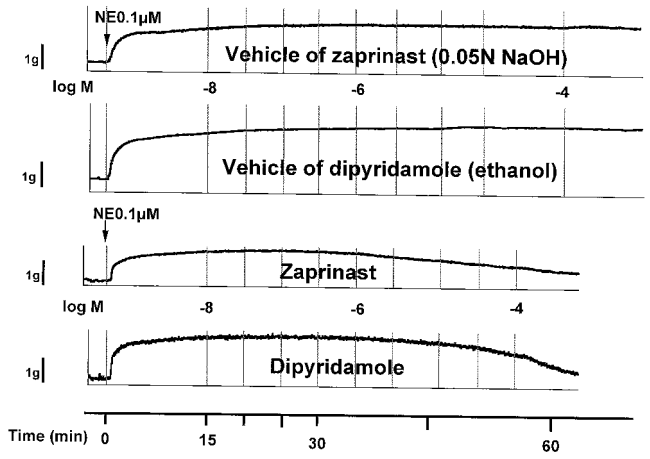

Figure 1. Original recording of response to zaprinast, dipyridamole or each vehicle on norepinephrine (NE, 0.1 μM) -induced vasoconstriction of pulmonary arteries from monocrotaline-treated rats. Vertical axis: constriction (g); Horizontal axis: time (min)

Relaxation

Both of zaprinast and dipyridamole showed a significant (p<0.0001) dose-dependency in dilation of PA from either monocrotaline or saline group (Figure 2). Dilation by zaprinast was significant at respective doses of 3.16 (p=0.0021), 10 (p=0.0005), 31.6 (p=0.0001) and 100 μM (p<0.0001) for PA from saline group, while it was significant at respective doses of 1 (p=0.0054), 3.16 (p=0.0002), 10 (p<0.0001), 31.6 (p<0.0001) and 100 μM (p<0.0001) for PA from monocrotaline group. Dilation by dipyridamole zaprinast was significant at respective doses of 31.6 (p=0.0032) and 100 μM (p

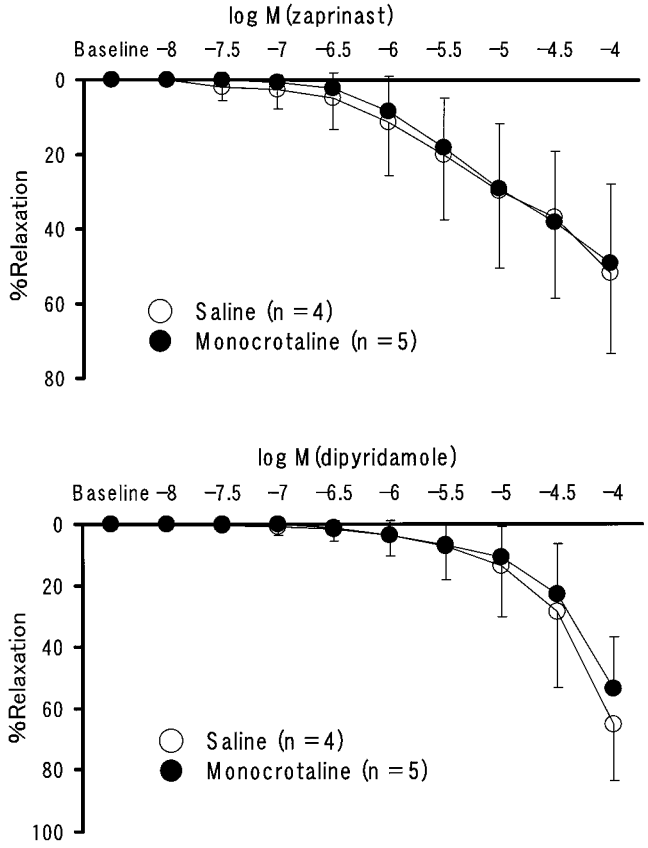

Figure 2. Effects of PDE V inhibitors on pulmonary arteries from saline- or monocrotaline-treated group. Each circle and each whisker denote mean and standard deviation, respectively. Open circle: saline-treated group; closed circle: monocrotaline-treated group. Relaxation is expressed as percent decrease in tension of that contraction induced by norepinephrine.

<0.0001) for PA from saline group, while it was significant at respective doses of 10 (p<0.0046), 31.6 (p=0.0010) and 100 μM (p <0.0001) for PA from monocrotaline group. No significant difference was observed between the monocrotaline and saline groups in the dilating effects of either zaprinast (p=0.7331) or dipyridamole (p=0.4341) (Figure 2).

Influence of NOS inhibitor

Pretreatment with L-NAME (0.1 mM) caused no additional effects on the resting tension (date not shown). The pretreatment of L-NAME significantly reduced the vasodilating effects of zaprinast, of 10 (p=0.0007), 31.6 (p=0.0004) and 100 μM (p=0.0005) on the PA from monocrotaline-treated group, while not on the PA from saline-treated group (p=0.1869, p=0.2674 and p=0.1630, respectively) (Figure 3). The pretreatment of L-NAME significantly reduced the vasodilating effects of dipyridamol of 100 μM (p= 0.0273), while not on the PA from saline-treated group (p=0.4009) (Figure 3).

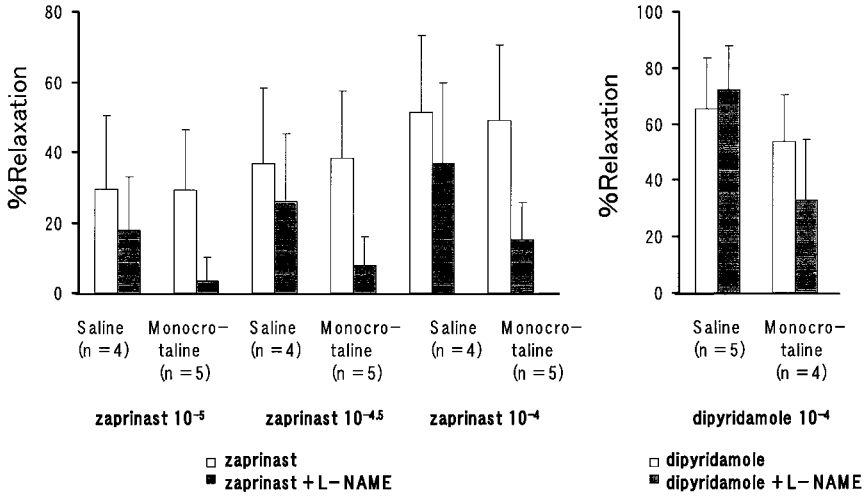

Figure 3. Effects of PDE V inhibitors on pulmonary arteries from saline- or monocrotaline-treated group with pretreatment of N^G-nitro-L-arginine methyl ester (L-NAME, 0.1 mM). Each bar and each whisker denote mean and standard deviation, respectively. Open bars: without L-NAME; closed bars: with L-NAME. Relaxation is expressed as percent decrease in tension of that contraction induced by norepinephrine.

Discussion

The results show that PDE V inhibitors exert the strong vasodilating effect on PA of pulmonary hypertensive rats as well as on that of normal rats. Since a NOS inhibitor reduced the vasodilating effect of zaprinast, NO would play a role in the effect of zaprinast on monocrotaline-induced pulmonary hypertensive rats.

The elevated pulmonary artery pressure reflects both pulmonary vasoconstriction and vascular structural remodelling, and it gives rise to right ventricular hypertrophy and right heart failure.[1] Administration of monocrotaline, a pyrrolizideine alkaloid found in the seeds and foliage of Crotalaria Spectabilis, to rats leads to the development of pulmonary hypertension and right ventricular hypertrophy.[12] The hepatic metabolite, dehydromonocrotaline, may be the reactive intermediate which causes pulmonary damage in spite of the very short half life.[13] It was shown that monocrotaline-induced pulmonary hypertension was associated with a reduced smooth muscle responsiveness to NO.[14] We did not measure pulmonary artery pressure or right ventricular pressure. However, our comparison of RV/(LV+S) ratio between monocrotaline and saline groups indicated that the right ventricular hypertrophy had indeed developed in monocrotaline group, suggesting that pulmonary hypertension had developed.

Regulation of cyclic nucleotide levels within a cell is the role of a group of hydrolytic enzyme, termed cyclic nucleotide PDE, which consists of several distinct isozymes.[15] Immunocytochemistry demonstrated that PDE V protein was localized to vascular smooth muscle.[5] Human pulmonary arteries contain four (I, III, IV, V) PDE isozyme families as identified by FPLC and isozyme inhibition by specific inhibitors,[3] and the inhibitors of PDE III, IV and V are known to relax preconstricted human pulmonary arteries. Thus, PDE I-V could play roles in the pulmonary arteries.

In the current study, we used zaprinast and dipyridamole as PDE V inhibitors, though they are not truly specific for PDE V. Zaprinast in micromolar concentrations also inhibits PDE I, PDE II and PDE IV at IC_{50}s one to two orders of magnitude greater than the IC_{50} for PDE V.[2] Zaprinast has also been used to enhance the vasodilator effect of inhaled nitric oxide in experimental pulmonary hypertension in lambs.[16] Dipyridamole attenuated increases in pulmonary artery pressure after NO withdrawal during cardiac surgery.[17] In addition to PDE V inhibition, dipyridamole is an adenosine reuptake inhibitor in endothelial cells and erythrocytes. As adenosine may directly and indirectly stimulate cyclic nucleotide production, this property of dipyridamole may complicate interpretation of its effect.[18]

Jeffery and Wanstall[19] demonstrated that the removal of the endothelium caused a shift of the concentration-response curve for zaprinast to a higher concentration range. McMahon et al.[20] demonstrated that the treatment with L-NAME significantly reduced the decrease in lobular arterial pressure caused by zaprinast in cat. Similarly, the present study demonstrated that pretreatment with L-NAME caused a significant reduction of vasodilation by zaprinast in monocrotaline-treated group, while not in saline-treated group. The reason of discrepancy in reduction between monocrotaline- and saline-treated groups was considered that there might be an increase in the tonic release of NO from the endothelium of the PA from monocrotaline-treated group, and thus pretreatment with NOS inhibitor in monocrotalone-treated group markedly exerted to reduce vasodilating effect by zaprinast than in saline-treated group. This suggests that NO would play a significant role in the vasodilating effect of zaprinast on monocrotaline-induced pulmonary hypertension.

Recently Surks et al.[21] have reported that cGMP-dependent protein kinase Iα mediates physiologic relaxation of vascular smooth muscle in response to nitric oxide and cGMP. Cohen et al.[2] found

that the concentration of cGMP in isolated lung perfusate was elevated nine-fold in hypertensive rats compared to normotensive control rats; 98% of lung cGMP hydrolytic activity was ascribed to cGMP-specific PDE V, without a significant decrease in PDE activity in hypertensive lungs, suggesting that the elevation in cGMP was due to accelerated production rather than reduced degradation. On the other hand, Takahashi et al.[22] found no difference in cGMP level in homogenized lung between pulmonary hypertensive rats induced by monocrotaline and control rats. Thus cGMP levels on PA from pulmonary hypertensive models have not yet been established.

In summary, zaprinast and dipyridamole exert strong vasodilating effect on PA of pulmonary hypertensive rats as well as that of normal rats, indicating that these compounds would be possible candidates to treat refractory pulmonary hypertension. Since a NOS inhibitor reduced the vasodilating effect of zaprinast, NO would play a role in the effect of zaprinast on monocrotaline-induced pulmonary hypertensive rats.

References

1. Wanstall JC. Pulmonary hypertension, pulmonary blood vessels and vasoactive drugs. *Proc West Pharm Soc* 40: 137-140, 1997
2. Cohen AH, Hanson K, Morris K et al. Inhibition of cyclic 3'-5'-guanosine monophosphate-specific phosphodiesterase selectively vasodilates the pulmonary circulation in chronically hypoxic rats. *J Clin Invest* 97: 172-179, 1996
3. Rabe KF, Tenor H, Dent G et al. Identification of PDE isozymes in human pulmonary artery and effect of selective PDE inhibitors. *Am J Physiol* 266: L536-L543, 1994
4. Gillespie PG, Beavo JA. Inhibition and stimulation of photoreceptor phosphodiesterases by dipyridamole and M&B 22,948. *Mol Pharmacol* 36: 773-781, 1989
5. Hanson KA, Ziegler JW, Rybalkin SD et al. Chronic pulmonary hypertension increase fetal lung cGMP phosphodiesterase activity. *Am J Physiol* 275: L931-L941, 1998
6. Goldstein I, Lue TF, Padma-Nathan H et al. Oral sildenafil in the treatment of erectile dysfunction. *N Engl J Med* 388:1397-1404, 1998
7. Ballard SA, Gingell CJ, Tang K et al. Effects of sildenafil on the relaxation of human corpus cavernosum tissue in vitro and on the activities of cyclic nucleotide phosphodiesterase isozymes. *J Urology* 159: 2164-2171, 1998
8. Atz AM, Wessel DL. Sildenafil ameliorates effects of inhaled nitric oxide withdrawal. *Anesthesiology* 91: 307-310, 1999
9. Wanstall JC, O'Donnell SR. Responses to vasodilator drugs on pulmonary artery preparations from pulmonary hypertensive rats. *Br J Pharmacol* 105: 152-158, 1992
10. Huraux C, Makita T, Montes F, Szlam F, Levy JH. A comparative evaluation of the effects of multiple vasodilators on human internal mammary artery. *Anesthesiology* 88: 1654-1659, 1998
11. SAS Institute. *SAS/Stat User's Guide, Version 6. 4th ed.* SAS Institute, Cary, NC, 1990
12. Werchan PM, Summer WR, Gerdes AM, McDonough KH. Right ventricular performance after monocrotaline-induced pulmonary hypertension. *Am J Physiol* 256: H1328-H1336, 1989
13. Pan LC, Wilson DW, Lame MW, Jones AD, Segall HJ. Cor pulmonale is caused by monocrotaline and dihydromonocrotaline, but not by glutathione or cysteine conjugates of dihydropyrrolizine. *Toxicol Appl Pharm* 118: 87-97, 1993
14. Prié S, Stewart DJ, Dupuis JD. Endothelin$_A$ receptor blockade improves nitric oxide-mediated vasodilation in monocrotaline-induced pulmonary hypertension. *Circulation* 97: 2169-2174, 1998
15. Beavo JA, Reifsnyder DH. Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors. *Trends Pharmacol Sci* 11: 150-155, 1990
16. Ichinose F, Adrie C, Hurford WE, Zapol WM. Prolonged pulmonary vasodilator action of inhaled nitric oxide by zaprinast in awake lambs. *J Appl Physiol* 78: 1288-1295, 1995
17. Ivy DD, Kinsella JP, Ziegler JW, Abman SH. Dipyridamole attenuates rebound pulmonary hypertension after inhaled nitric oxide withdrawal in postoperative congenital heart disease. *J Thorac Cardiov Sur* 115: 875-882, 1998
18. Ziegler JW, Ivy DD, Fox JJ, et al. Dipyridamole, a cGMP phosphodiesterase inhibitor, causes pulmonary vasodilation in the ovine fetus. *Am J Physiol* 269: H473-H479, 1995
19. Jeffery TK, Wanstall JC. Phosphodiesterase III and V inhibitors on pulmonary artery from pulmonary hypertensive rats: differences between early and established pulmonary hypertension. *J Cardiovasc Pham* 32: 213-219, 1998
20. McMahon TJ, Ignarro LJ, Kadowitz PJ. Influence of zaprinast on vascular tone and vasodilator responses in the cat pulmonary vascular bed. *J Appl Physiol* 74: 1704-1711, 1993
21. Surks HK, Mochizuki N, Kasai Y et al. Regulation of myosin phosphatase by a specific interaction with cGMP-dependent protein kinase Iα. *Science* 286: 1583-1587, 1999
22. Takahashi T, Kanda T, Inoue M et al. A selective type V phosphodiesterase inhibitor, E4021, protects the development of right ventricular overload and medial thickening of pulmonary arteries in a rat model of pulmonary hypertension. *Life Sci* 59: 371-377, 1996