Effect of Diltiazem on Hypoxic Pulmonary Vasoconstriction in Dogs

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We have examined the effect of diltiazem upon the pulmonary vascular response to the left lower lobe (LLL) hypoxia in dogs.

Without diltiazem, the fraction of cardiac output perfusing the LLL (Q_{LLL}/Q_T) measured by the ultrasonic doppler rheometer in the hypoxic phase was 21.0 ± 11.7(%) of the ratio in the first normoxic phase. When diltiazem was given as a 0.48 mg/kg intravenous bolus followed by an intravenous infusion of 0.48 mg/kg/hr and 0.96 mg/kg intravenous bolus followed by an intravenous infusion of 0.96 mg/kg/hr, Q_{LLL}/Q_T in the hypoxic phase were 34.0 ± 14.0, 48.6 ± 16.1(%) of the ratio in the first normoxic phase respectively. Significant difference was observed at all diltiazem concentrations.

With respect to Pa_{O_2}, significant difference was not observed at all diltiazem concentrations in the ratio of the hypoxic phase to the first control phase.

So we concluded that diltiazem obviously attenuated hypoxic pulmonary vasoconstriction (HPV) response but did not decrease Pa_{O_2} because of keeping myocardial oxygen balance and better ventilation/perfusing relationship. (Key words: diltiazem, calcium-antagonist, doppler rheometer, hypoxia, hypoxic pulmonary vasoconstriction (HPV))

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HPV is widely known as a mechanism which counteracts arterial hypoxemia by diversion of pulmonary blood flow from poorly to more properly oxygenated areas of the lungs[1]. However, the mechanism by which alveolar hypoxia acts within the lung to elicit pulmonary arterial vasoconstriction is unknown. So it is very important in two respects to study the effects of calcium-antagonists to HPV. First, based on current understanding of the roles of extra- and intracellular calcium ions in excitation-constriction coupling of vascular smooth muscle[2,3], HPV might involve membrane depolarization and transmembrane influx of extracellular calcium, or transmitter such as catecholamine or histamine-induced release of calcium from an intracellular pool or both. Second, calcium-antagonists are really useful or not to treat of angina pectoris, hypertension, cardiac tachyarrhythmias.

So we studied the hemodynamics and blood gas effects of one of the new calcium channel blocking drugs, diltiazem to test whether diltiazem attenuates pulmonary vasoconstriction associated with alveolar hypoxia in dogs.

Materials and Methods

a) *Anesthesia and Operation*

Six female mongrel dogs (7.1 ± 1.4 kg) were anesthetized with 30 mg/kg of pento-

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barbital (supplemented with 25–50 mg/hr – Truth A-2 No 6630) and 0.2 mg/kg of pancuronium (supplemented with 0.5 mg when spontaneous respiration appeared). A tube of 9.5 mm inner diameter was inserted into the trachea and the lungs were ventilated with 100% oxygen. A 20G plastic needle was put into the femoral artery to be used for sampling arterial blood and monitoring of arterial pressure. A 7 Fr Swan-Ganz catheter was inserted into the internal jugular vein and was used for sampling mixed venous blood and pulmonary arterial pressure monitoring. A 18G central venous catheter was also inserted into the same side internal jugular vein for measurement of the right atrial pressure. Then, a leftsided thoracotomy was performed at the fourth intercostal space and the left auricle was cannulated through the left atrium for measurement of the left atrial pressure. Pressures were recorded with a Hewlett-Packard monitoring system (HP78342 Monitor with HP1290A quartz pressure transducer) by adjusting the reading at the mid-cardiac level to zero by in vivo calibration. Blood flow was determined by means of an ultrasonic transit time rheometer (Transonic T101, ADVANCE CO., LTD.) which was attached to the main pulmonary artery (mPA), and to the left lower lobe (LLL) artery employing flow probes of an internal diameter of 12 and 3 mm, respectively. Blood flow in the mPA was regarded as equal to the cardiac output (Q_T), and the blood flow distribution to the LLL was expressed as a fraction of cardiac output (Q_{LLL}/Q_T). A 14 Fr endotracheal tube was inserted into the bronchus of the LLL and fixed there with the central end of the bronchus completely ligated. The residual lung (RL) was ventilated (Harvard NSH-34RH) with pure oxygen for the whole experimental period with 5 cmH_2O PEEP. The LLL was not ventilated with pure oxygen, as was the residual lung. 2 L/min flow rate (Igarashi precision membrane flow meter F-1) with 100% oxygen or anoxic mixed gas($95\%N_2 + 5\%CO_2$) was applied to LLL under 5 cmH_2O CPAP with a water seal. A change in the gas composition in LLL was preceded by 20 ventilations (tidal volume is about 200 ml) to provided a homogeneous gas mixing prior each sequence. RL ventilation was started under conditions of 20 ml/kg, 15 times/min, and was controlled later so that end-tidal CO_2 (DATEX CD300 CO_2 ANALIZER) was kept between 35 and 45 mmHg.

Physiologic saline was continuously infused at a rate of 50–100 ml/hr. Sodium bicarbonate was infused i.v. to correct any metabolic acidosis. Blood temperature was monitored and maintained at 37 ± 2°C with the use of blankets and table lamps.

b) Experimental Protocol

Prior to the investigational period, LLL was exposed to three challenges, each lasting 15 min interrupted by 15 min of 100% oxygen administration to achieve maximum HPV.

After that, we studied the effects on LLL hemodynamics of the three different diltiazem concentrations. Each experiment consisted of three sequences, a control period of LLL O_2 administration (C1 phase) lasting 1 hr, a period of LLL anoxic mixed gas administration (H phase) and second control period of LLL O_2 administration (C2 phase), each lasting 30 min.

The first experiment was without diltiazem, and the last two experiment were with diltiazem. During the 2nd experiment (8μ experiment), diltiazem, 0.48 mg/kg intravenous bolus followed by an intravenous infusion of 0.48 mg/kg/hr (8 μg/kg/min) was administered with a infusion pump (Truth A-2, No 6630). In the same way, during the 3rd experiment (16μ experiment), the drug, 0.96 mg/kg i.v. bolus followed by an intravenous infusion of 0.96 mg/kg/hr (16 μg/kg/min) was administered.

After each sequence, blood flow in the mPA and in LLL artery were determined as well as heart rate, systemic blood pressure, pulmonary artery pressure, central venous pressure and left atrial pressure.

Systemic arterial and pulmonary blood sampling were drawn for blood gas analysis and serum diltiazem concentration measurement. Arterial and mixed venous oxy-

Table 1. Effects of hypoxia and diltiazem infusion on blood gases

Variables	Phases	Diltiazem 0r	Diltiazem 8r	Diltiazem 16r
pH	C1	7.328 ± 0.051	7.343 ± 0.048	7.302 ± 0.053
	H	7.344 ± 0.040	7.340 ± 0.038	7.317 ± 0.064
	C2	7.339 ± 0.039	7.314 ± 0.041	7.330 ± 0.070
Pa_{CO_2} (mmHg)	C1	39.2 ± 5.3	36.9 ± 3.0	38.9 ± 5.0
	H	38.1 ± 4.0	36.2 ± 3.4	37.9 ± 3.2
	C2	37.7 ± 3.2	38.4 ± 4.7	37.6 ± 3.5
Pa_{O_2} (mmHg)	C1	434.9 ± 43.9	454.3 ± 72.3	382.2 ± 72.4
	H	321.7 ± 69.4*	295.4 ± 71.6*§§	209.5 ± 31.4*§§§
	C2	424.5 ± 80.7**	419.4 ± 71.3**	391.2 ± 101.9**
Pv_{O_2} (mmHg)	C1	57.6 ± 6.6	60.0 ± 6.4	59.6 ± 6.9
	H	54.9 ± 5.1*	55.8 ± 6.3	50.9 ± 2.9*
	C2	57.8 ± 6.9**	59.5 ± 7.6	57.4 ± 10.4
Qs/Qt	C1	16.2 ± 3.7	14.9 ± 4.5	20.0 ± 5.3
	H	22.7 ± 5.6*	23.7 ± 6.1*	27.3 ± 5.3*
	C2	16.4 ± 5.5**	16.6 ± 3.9**	18.4 ± 4.0**

Abbreviations: Qs/Qt = shunt ratio, Values are mean ± SD
*$P < 0.05$ significance between C1 and H
**$P < 0.05$ significance between H and C2
§§$P < 0.05$ significance between 8r and 16r
§§§$P < 0.05$ significance between 16r and 0r

gen and carbon dioxide tensions and pH, were measured by standard method (ABL-2 analyzer, Radiometer Co.). Hemoglobin concentration and oxygen saturation of the blood were measured by spectrophotometry (OSM-2, Radiometer Co.). The electrodes were calibrated with known gases and buffer solutions before each administration. The serum diltiazem concentrations were measured by means of high performance liquid chromatography (LC-6A, Shimadzu Seisakusho)[4]. The shunt ratio was calculated by Hill's equation[5].

c) *Statistics*

Analysis of variance (ANOVA) was employed in a statistical comparison between the concentrations of diltiazem, while paired t-test was used in statistical comparison between each phase, the differences being labeled as significant at $P < 0.05$ invariably.

Results

The serum concentration of diltiazem was 109 ± 31 ng/ml during 8μ experiment, and 276 ± 96 ng/ml during 16μ experiment. There were no significant differences among each of the first control phase (C1 phase), hypoxic phase (H phase), and the second control phase (C2 phase).

Effects of hypoxia and diltiazem infusion on blood gases and hemodynamics were summarized in table 1, 2.

No significant differences were observed in pH, arterial CO_2 tension, pulmonary artery pressure, right and left atrial pressure and pulmonary vascular resistance of residual lung (PVR_{RL}) among each of the C1, H, C2 phases and among each concentration of diltiazem.

Q_{LLL}/Q_T, pulmonary vascular resistance of left lower lobe (PVR_{LLL}), Pa_{O_2} and shunt ratio (Qs/Qt) significantly changed in the H phase and restored the level of the C1 phase in the C2 phase. No significant difference was observed in the C1 phase among different concentrations of diltiazem. The higher diltiazem concentration was, the higher value in Q_{LLL}/Q_T and the lower value in PVR_{LLL} were observed in the H phase. With respect to Pa_{O_2}, significant difference was observed among concentrations of diltiazem in the H phase Pa_{O_2}. However, significant difference

Table 2. Effects of hypoxia and diltiazem infusion on hemodynamics

Variables	Phases	Diltiazem		
		0r	8r	16r
Q_{LLL}/Q_T (%)	C1	22.4 ± 4.8	21.2 ± 3.1	20.8 ± 4.7
	H	4.8 ± 2.7*	7.3 ± 3.3*	10.1 ± 3.7*
	C2	22.9 ± 6.1**	22.0 ± 3.6**	22.1 ± 5.8**
CO (L/min)	C1	0.56 ± 0.10	0.63 ± 0.17	0.62 ± 0.21
	H	0.55 ± 0.11	0.55 ± 0.12	0.53 ± 0.08
	C2	0.58 ± 0.16	0.61 ± 0.15	0.56 ± 0.18
HR (beats/min)	C1	163 ± 23	152 ± 17	135 ± 17
	H	165 ± 24	150 ± 21	134 ± 17
	C2	163 ± 24	149 ± 20	132 ± 18
MAP (mmHg)	C1	119 ± 8§	103 ± 13§§	90 ± 15§§§
	H	120 ± 8§	100 ± 13§§	87 ± 12§§§
	C2	120 ± 11§	102 ± 13§§	86 ± 11§§§
PAP (mmHg)	C1	17 ± 2	18 ± 2	18 ± 2
	H	18 ± 3	18 ± 2	19 ± 3
	C2	17 ± 3	18 ± 2	18 ± 2
LAP (mmHg)	C1	7 ± 3	8 ± 2	8 ± 1
	H	7 ± 2	8 ± 2	8 ± 1
	C2	7 ± 2	8 ± 1	8 ± 1
RAP (mmHg)	C1	5 ± 2	6 ± 2	8 ± 3
	H	6 ± 2	7 ± 2	8 ± 3
	C2	5 ± 1	7 ± 3	7 ± 4
SVR	C1	16668 ± 2989§	12849 ± 2980	11320 ± 3202§§§
(dyne·cm·sec^{-5})	H	17217 ± 3080§	13993 ± 2452	12361 ± 2628§§§
	C2	16996 ± 4132§	13193 ± 3434	11932 ± 2852§§§
PVR_{RL}	C1	1916 ± 1172	1754 ± 615	1844 ± 666
(dyne·cm·sec^{-5})	H	1690 ± 761	1755 ± 507	1820 ± 502
	C2	2034 ± 1093	1784 ± 658	2175 ± 1007
PVR_{LLL}	C1	6189 ± 2548	6259 ± 1522	6872 ± 2235
(dyne·cm·sec^{-5})	H	51587 ± 39781*	30561 ± 21642*	20067 ± 11768*
	C2	6463 ± 1971**	6131 ± 1929**	7269 ± 2159**
SV (ml/beat)	C1	3.5 ± 0.7	4.2 ± 1.1	4.6 ± 1.3
	H	3.4 ± 0.7	3.7 ± 0.8	3.9 ± 0.5
	C2	3.6 ± 0.9	4.1 ± 0.9	4.2 ± 0.1
RPP (beat·mmHg)	C1	23355 ± 3684	19906 ± 2761	16109 ± 2567
	H	23862 ± 4079	19079 ± 3270	15509 ± 2449
	C2	23763 ± 4136	19190 ± 2756	15180 ± 2306

Abbreviations: Q_{LLL}/Q_T = blood flow of left lower lobe as percent of total lung flow; CO = cardiac output, blood flow of main pulmonary artery; HR = heart rate; MAP = mean arterial pressure; LAP = left atrial pressure; RAP = right atrial pressure; SVR = systemic vascular resistance; PVR_{RL} = pulmonary vascular resistance of residual lung. PVR_{LLL} = pulmonary vascular resistance of left lower lobe. Values are mean ± SD.

*$P < 0.05$ significance between C1 and H

**$P < 0.05$ significance between H and C2

§$P < 0.05$ significance between 0r and 8r

§§$P < 0.05$ significance between 8r and 16r

§§§$P < 0.05$ significance between 16r and 0r

was not observed at all diltiazem concentrations in the ratio of the H phase Pa_{O_2} to the C1 phase Pa_{O_2}. No significant difference was observed in Qs/Qt among concentrations of diltiazem.

Cardiac output (CO) and stroke volume (SV) increased at the 8μ experiment and more increased at the 16μ experiment. However, no significant difference was observed among each of the C1, H, C2 phases and among each concentrations of diltiazem.

Heart rate (HR) and rate pressure product (RPP) decreased at the 8μ experiment and more decreased at the 16μ experiment. However, no significant difference was observed among each of the C1, H, C2 phases and among each concentrations of diltiazem.

Mean arterial pressure (MAP) significantly decreased at the 8μ experiment and more decreased at the 16μ experiment. Significant difference was not observed among each of the C1, H and C2 phase.

Discussion

Calcium-antagonists have been extensively applied in the treatment of hypertension, angina pectoris, and cardiac tachyarrhythmias in the past decade.

Regardless of the mechanism of subcellular action, the demonstration that calcium-antagonists work locally within the lung to attenuate hypoxic pulmonary vasoconstriction[6–9] may be considerably important in predicting any ultimate benefit to patients with pulmonary hypertension given the agent as therapy. The pharmacologic profile of calcium channel blocking drugs show them to be also advantageous for favorable manipulation of the myocardial oxygen balance[10]. So calcium channel blockers are widely used to protect the myocardium for ischemic damage because of their unique acts on blood vessels and/or cardiac tissue.

However, some calcium channel blockers have disadvantage of no improvement in pulmonary blood flow and gas exchange with local hypoxic manipulation[11].

Bishop at al. found that nifedipine increased venous admixture[11]. However, Simonneau et al. found only a slight (45 ± 2 to 42 ± 2 mmHg) although statistically significant change in arterial P_{O_2} in a group of 13 patients with acute respiratory failure given nifedipine[12], and Muramoto et al. showed no change in arterial P_{O_2} either at rest or during exercise in 9 subjects with chronic obstructive pulmonary disease. given the agent[13]. This lack of clinically significant desaturation after the administration of nifedipine in man might be explained on the basis of an increase in cardiac output and a decrease in arteriovenous oxygen content, as occurred in Muramoto's patients, were it not for the fact that mixed venous P_{O_2} did not change in subjects studied by Simonneau et al. Kennedy et al. reported that the ventilation perfusion relations that follow nifedipine administration may be unusually complex. Nevertheless, these agents offer promise in the treatment of patients with cor pulmonale in which low cardiac output is a factor limiting clinical well-being[6].

In the present study, Q_{LLL}/Q_T had difference among concentrations of diltiazem. However, the ratio of Pa_{O_2} in the hypoxic phase to Pa_{O_2} in the first control phase had insignificant difference among concentrations of diltiazem. Shunt ratio (Qs/Qt) as an index of ventilation/perfusion relationships were as the same way. This may be explained on the basis of increasing tendency in cardiac output, a decrease in arteriovenous oxygen content and moreover a decrease in rate pressure product, or reductions in myocardial oxygen consumption. After all, diltiazem has obviously inhibitory effect on hypoxic pulmonary vasoconstriction. However, diltiazem dose not influence the arterial oxygen tension to a certain extent. We do not see from this study that how much the hypoxic pulmonary vasoconstriction is inhibited influences arterial oxygenation.

In our study, serum diltiazem concentrations were 109 ± 31 ng/ml during 8μ experiment and 276 ± 96 ng/ml during 16μ experiment.

Fujimoto at al. showed that significant electrophysiologic effects appeared at 0.02 mg/kg/min infusion (312 ± 165 ng/ml

plasma concentration), and at a dose when antiarrhythmic effects are evident, the safety of diltiazem is corroborated by lack of adverse hemodynamic effects[14]. Kawai et al. reported that diltiazem and verapamil exert a similar suppressive effect on the atrioventricular (AV) node and are useful for treating and preventing AV nodal reentrant tachycardia. However, nifedipine, in clinically practical doses, has no antiarrhythmic properties, probably because of reflex activation of the sympathetic system secondary to its hypotensive effect, which is greater than that of the other two calcium-antagonists[15]. This report and our blood gas data elicit that diltiazem has antiarrhythmic properties and little hemodynamic effects at about 300 ng/ml plasma concentration or one of the clinically beneficial points for diltiazem.

Takeda et al. reported that verapamil and nifedipine produced a dilatation only of the small resistive artery, however diltiazem produced a dilatation both of the small resistive artery and the large conductance artery, the dilatation of the latter being of a little longer duration[16]. This is also beneficial point of diltiazem for reducing the myocardial oxygen balance.

In the present study, our model has two characteristic point. First, LLL was not ventilated but was kept under CPAP. One problem for no ventilation is the different endtidal P_{CO_2} -values in two separated parts of the lung or the difference in ventilation mechanics. In other words, LLL P_{CO_2} will be higher than RL P_{CO_2} because the mixed venous/alveolar P_{CO_2} gradient will be less in LLL than in RL. Regional PVR will increase with an increase in end-tidal P_{CO_2} and that may increase the response of HPV. But this will not make it impossible to compare HPV responses among different diltiazem concentrations. Clinically hypoxic region was not ventilated. This is why we employed CPAP-system instead of ventilating the LLL.

Second, we measured the blood flows by the ultrasonic doppler rheometer instead of an electromagnetic flow meter. In terms of stability in circulatory condition, it was desirable that the cardiac output was measured directly by attaching the probes to the main pulmonary artery instead of using thermodilution method with a Swan-Ganz catheter. The electromagnetic rheometer has been often used for the measurement of blood flow. However, there have been problems in preparation for an experiments with this device; a flow probe must be chosen precisely depending on the diameter of blood vessels to be examined, and measurement values could vary depending on the extent to which the vessel is detached from connective tissue surrounding it. Therefore, we used an ultrasonic transit time rheometer for blood flow measurement in this experiment. This type of rheometer enabled us to measure the flow rate directly regardless of the diameter of blood vessel. It is important whether the measurement is based upon a continuous and stable determination of the transsectional area of the vessels. This flow probe has advantages in that it can correct aberrations in attachment angles by 2 return-sampling and that an adoption of free bracket has resolved the problem of deviation caused by pressure. These enable the continuous and stable determination.

In conclusion, calcium-antagonists' properties are very different. However, diltiazem dose not decrease arterial oxygen tension to a certain extent and is more beneficial for hypertension, angina pectoris, and cardiac tachyarrhythmias although diltiazem obviously attenuates hypoxic poulmonary vasoconstriction (HPV) because of keeping myocardial oxygen balance and better ventilation/perfusion relationship among calcium-antagonists.

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