

Theophylline-Induced Left Ventricular Relaxation Disturbance in Magnesium-Deficient Rats: Improvement by K201, a Novel 1,4-Benzothiazepine Derivative

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Background: Although magnesium deficiency induces left ventricular dysfunction, it is not known whether both systolic and diastolic functions are altered to the same extent. In this study, we investigated the effects of theophylline on left ventricular function in rats fed a normal diet or a magnesium-deficient diet for 1 month, and determined whether K201, a multi-channel blocker, modulated the effects of theophylline.

Methods: Theophylline was infused at 5 mg/kg/min for 15 min in 6 control rats and 6 magnesium-deficient rats, and hemodynamic measurements were performed. In another 6 magnesium-deficient rats, K201 was infused at 0.1 mg/kg/min for 15 min simultaneously with theophylline.

Results: Theophylline induced persistent increases in heart rate, peak positive first derivative of left ventricular pressure (+dP/dt), and a transient increase in left ventricular end-diastolic pressure (LVEDP), but did not affect left ventricular systolic pressure (LVSP) and peak negative first derivative of left ventricular pressure (-dP/dt) in the control rats. In contrast, in the magnesium-deficient rats, there was a persistent decrease in LVSP and a persistent increase in -dP/dt after theophylline infusion, although increases in heart rate, +dP/dt and LVEDP were similar to those in the control rats. When K201 was infused along with theophylline in the magnesium-deficient rats, both the decrease in LVSP and increase in -dP/dt were suppressed.

Conclusions: Theophylline impaired left ventricular function in the magnesium-deficient rats, and this was improved by K201. K201 may provide new insights regarding future strategies for heart failure treatment.

Key Words: K201 • Left ventricular function • Left ventricular relaxation • Magnesium • Theophylline

INTRODUCTION

Magnesium is an important chemical element for maintaining cell structure, and it is known to act as a co-

factor in several enzymatic reactions that are essential for maintaining normal homeostasis. Although it has been suggested that magnesium intake is generally lower than the recommended amount, relatively few studies have investigated the clinical effects of magnesium deficiency. A relationship between magnesium and cardiovascular diseases was first identified in 1970. It is known that serum magnesium level is reduced in patients with coronary artery disease and that intramyocardial magnesium content is reduced in patients with myocardial infarction or sudden death.¹⁻⁴ Experimentally, a low magnesium state induces focal myocardial necrosis and fi-

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brosis which can lead to cardiomyopathy, and magnesium administration has been shown to protect the myocardium.⁵ Although magnesium deficiency has been shown to induce left ventricular dysfunction via impairment of myocardial energy metabolism, excitation-contraction coupling and neurohormonal balance,⁶ it is not known whether systolic and diastolic function are altered to the same extent.

Xanthine derivatives such as caffeine and theophylline inhibit phosphodiesterase and increase cyclic adenosine monophosphate.^{6,7} In addition, they have been shown to decrease ryanodine receptor activity and promote Ca^{2+} release from the sarcoplasmic reticulum, leading to positive inotropic and chronotropic actions.^{8,9}

K201 (JTV-519) is a 1,4-benzothiazepine derivative that has been developed as a drug with a stronger myocardial protective effect than Ca^{2+} channel blockers or β -adrenoreceptor blockers, but with extremely weak negative inotropic and chronotropic actions.¹⁰ K201 has been shown to have multiple effects on cardiac function since it blocks multiple channels including Ca^{2+} and K^+ channels,¹¹ protects against myocardial ischemic-reperfusion injury, and stabilizes cardiac ryanodine receptors,¹² resulting in inhibition of Ca^{2+} spark or Ca^{2+} release from the sarcoplasmic reticulum.^{13,14}

In this study, we investigated the effects of theophylline on left ventricular function, especially left ventricular diastolic function during magnesium deficiency, and determined whether K201 modulates these effects.

METHODS

Experimental protocol

Wistar male rats ($n = 48$) aged 5-6 weeks (body weight 200 g) were used in this study. The study was conducted in accordance with the animal ethics code of Dokkyo Medical University, after approval by the University Animal Ethics Committee. A control diet (52 mg magnesium/100 g feed; $n = 24$) or magnesium-deficient diet (2 mg magnesium/100 g feed; $n = 24$) (Oriental Yeast Co. Ltd., Tokyo, Japan) was given for 1 month. All animals were allowed to drink distilled water. After 1 month, the animals were endotracheally intubated with a polyethylene tube (SP102, Natsume Seisakusho Co. Ltd., Tokyo, Japan), anesthetized with 3% isoflurane (Merck KGaA,

Darmstadt, Germany), and mechanically ventilated with a tidal volume of 3 ml at a rate of 60/min using a ventilator (SN-48-7, Shinano Manufacturing Co. Ltd., Tokyo Japan). A micromanometer-tipped pressure catheter (2F SPC-320, Millar Instruments Inc., Houston, Texas) was inserted into the right cervical artery and advanced to measure aortic pressure and left ventricular pressure. A polyethylene tube was inserted into the right femoral vein, and test solutions were injected using a continuous injector. Electrocardiography (ECG) apparatus was attached to the extremities of the animals, and lead II was monitored. The ECG and pressure curve data were stored on a personal computer (Power Lab, AD Instruments, Ltd., Sydney, Australia). After the blood pressure and heart rate had stabilized, theophylline (Sigma, St Louis, Missouri) in distilled water was infused at 5 mg/kg/min for 15 min into 6 control rats and 6 magnesium-deficient rats. The dose of theophylline (5 mg/kg/min) was chosen as that which produced the maximum decrease in left ventricular relaxation among doses of 1, 2.5, 5, and 7.5 mg/kg/min in a preliminary experiment. In another 6 magnesium-deficient rats, K201 (Aetas Pharma Co., Ltd, Tokyo, Japan) was infused at 0.1 mg/kg/min for 15 min simultaneously with theophylline.

Measurement of serum markers

In all 24 rats with the control diet and 24 rats with the low magnesium diet, serum concentrations of magnesium (xylydyl blue method) and calcium (Arsenazo III method) were measured. In the 6 control rats, the 6 magnesium deficient rats and the 6 magnesium-deficient rats that received K201, serum creatine kinase (CK: ultraviolet method) levels were measured 3 hours after theophylline infusion.

Hemodynamic measurements

In all 24 control rats and 24 magnesium-deficient rats, heart rate, left ventricular systolic pressure (LVSP), double product (calculated as heart rate \times LVSP) and left ventricular end-diastolic pressure (LVEDP) were measured. Left ventricular pressure was differentiated, and the peak positive and peak negative first derivative (peak $+dP/dt$) and (peak $-dP/dt$) were measured simultaneously. In the 6 control rats, 6 magnesium-deficient rats, and 6 magnesium-deficient rats that received K201, these hemodynamic measurements were performed at base-

line before the start of theophylline infusion, 15 min (at the time when the theophylline infusion was terminated), 40 min and 60 min after the start of theophylline infusion.

Statistical analysis

Values are shown as mean ± standard deviation. For continuous variables, intergroup comparisons for were performed using an unpaired t-test. Multiple group comparisons were performed using one-way analysis of variance (ANOVA) followed by a Tukey post-hoc test. Serial changes in the values were analyzed using two-factor repeated measures ANOVA. A p value less than 0.05 was considered to be statistically significant.

RESULTS

At the time point just before the experiment was performed, i.e., 1 month after the control diet or the magnesium-deficient diet had been started, the body weight was similar between the control rats (n = 24) and magnesium-deficient rats (n = 24) (302 ± 12 vs. 306 ± 16 g). Table 1 shows comparisons of serum levels of magnesium and calcium and hemodynamic parameters between the control rats and magnesium-deficient rats. The serum magnesium level was significantly lower and the serum calcium level was significantly higher in the magnesium-deficient rats compared with the control

Table 1. Comparisons of serum magnesium and calcium levels, and hemodynamic parameters between control rats and magnesium-deficient rats

	Control rat (n = 24)	Magnesium deficient rat (n = 24)	p value
Magnesium (mg/dL)	2.2 ± 0.2	0.6 ± 0.2	< 0.001
Calcium (mg/dL)	10.8 ± 0.2	12.2 ± 0.6	< 0.01
Heart rate (/min)	331 ± 39	319 ± 57	NS
LVSP (mmHg)	95 ± 8	91 ± 10	NS
Double product (beat × mmHg)	3170 ± 5602	2861 ± 8105	NS
LVEDP (mmHg)	6.4 ± 1.3	6.8 ± 1.9	NS
Peak +dP/dt (mmHg/sec)	5780 ± 1131	4778 ± 1375	< 0.01
Peak -dP/dt (mmHg/sec)	-4681 ± 825	-4496 ± 1287	NS

dP/dt, first derivative of left ventricular pressure; LVEDP, left ventricular end diastolic pressure; LVSP, left ventricular systolic pressure.

rats. Although peak +dP/dt was significantly lower in the magnesium-deficient rats compared with the control rats, the other hemodynamic parameters were similar between the two groups.

Serial changes in hemodynamic parameters after theophylline infusion in the control rats (n = 6) and magnesium-deficient rats (n = 6) are shown in Figure 1 and Figure 2, respectively. The heart rate significantly increased at 15 min (at the time when the theophylline infusion was terminated), and the elevated rate continued until 40-60 min after the start of theophylline infusion in

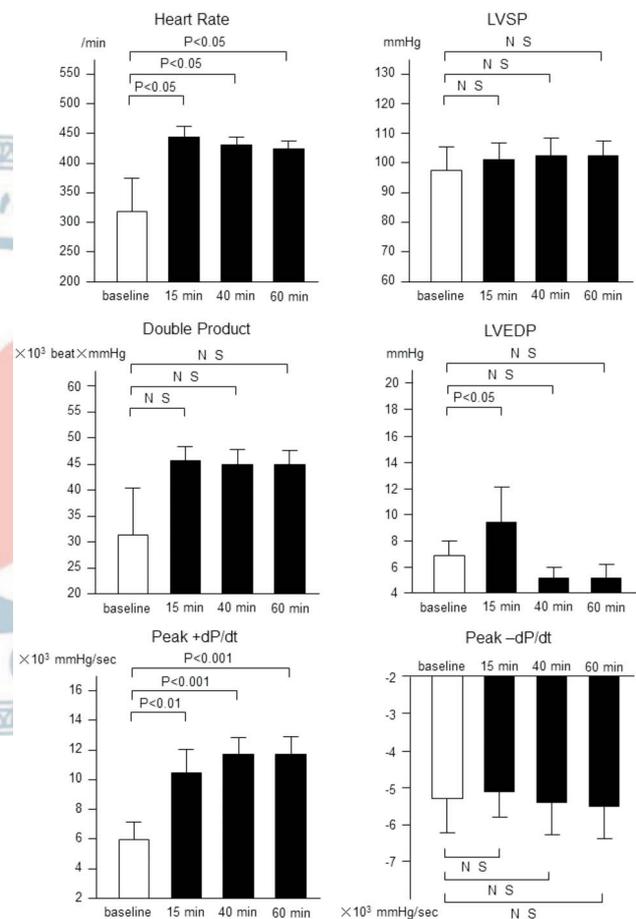


Figure 1. Serial changes in hemodynamic parameters after theophylline infusion in the control rats (n = 6). The heart rate significantly increased at 15 min and the elevated level was continued to 40-60 min after start of theophylline infusion. The LVEDP increased transiently at 15 min but returned to the baseline level at 40-60 min. The peak +dP/dt began to increase at 15 min and the elevated level was continued at 40-60 min. The LVSP, double product and peak -dP/dt did not change significantly during the observation period. dP/dt, first derivative of left ventricular pressure; LVEDP, left ventricular end diastolic pressure; LVSP, left ventricular systolic pressure.

both groups. The LVSP did not change significantly in the control rats, but decreased significantly at 15 min and remained decreased at 40-60 min in the magnesium-deficient rats. The double product did not change significantly in either group. The LVEDP increased transiently at 15 min, but returned to the baseline level at 40-60 min in both groups. The peak +dP/dt began to increase significantly at 15 min, and remained elevated at 40-60 min in the control rats, whereas it did not change at 15 min in the magnesium-deficient rats but increased significantly at 40-60 min. The peak -dP/dt did not change significantly in the control rats, but it increased significantly at 15-60 min in the magnesium-deficient rats.

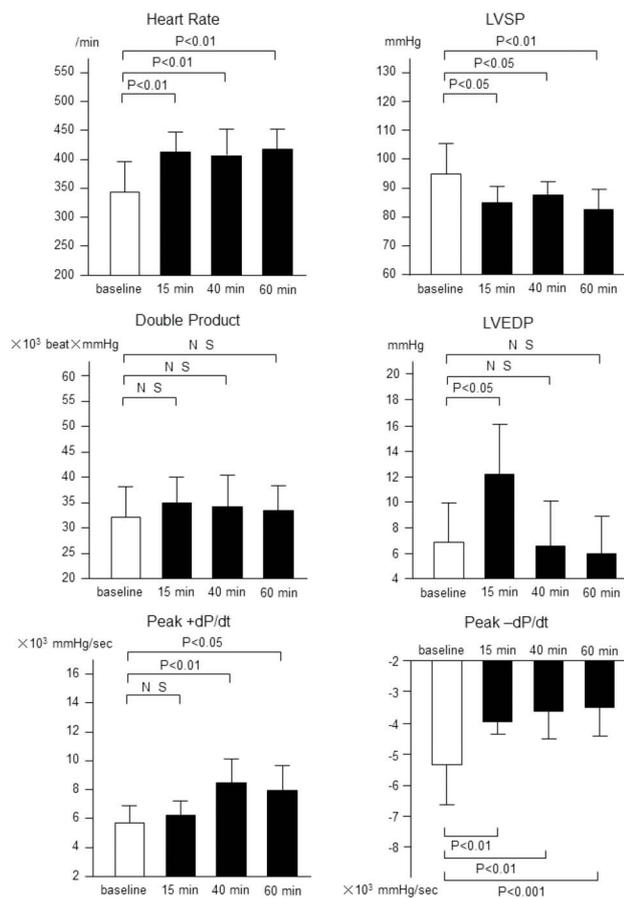


Figure 2. Serial changes in hemodynamic parameters after theophylline infusion in the magnesium-deficient rats (n = 6). The heart rate significantly increased at 15 min and the rate remained elevated from 40-60 min after the start of the theophylline infusion. The LVSP decreased at 15 min and the decreased level was continued to 40-60 min. The double product did not change. The LVEDP increased transiently at 15 min but returned to the baseline level at 40-60 min. The peak +dP/dt did not change at 15 min, but increased significantly at 40-60 min. The peak -dP/dt increased at 15-60 min.

In the magnesium-deficient rats, serial changes in heart rate, LVEDP and peak +dP/dt after infusion of theophylline and K201 (n = 6) were similar to those of theophylline alone (n = 6). However, the changes in LVSP and peak -dP/dt were significantly attenuated when K201 was also added compared with the infusion of theophylline alone. The double product increased in a time dependent manner when K201 was added, whereas it did not change after the infusion of theophylline alone (Figure 3).

The CK level 3 hours after theophylline infusion was higher in the magnesium-deficient rats compared with the control rats (375 ± 95 vs. 155 ± 100 U/L, p < 0.01). In the magnesium-deficient rats, the CK level after the infusion of theophylline and K201 (250 ± 85 U/L) was lower than that after the infusion of theophylline alone (p < 0.05).

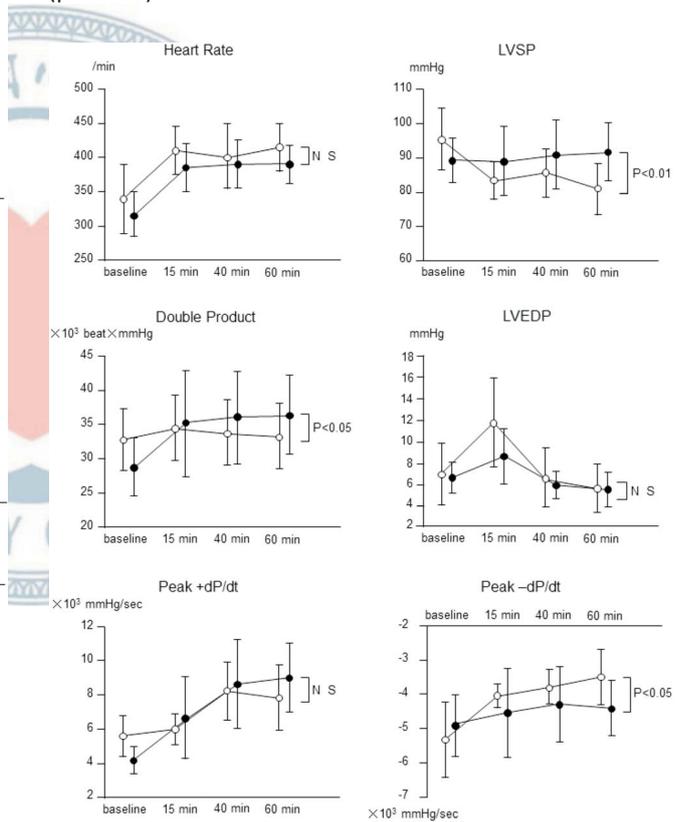


Figure 3. Comparison of serial changes in hemodynamic parameters between infusion of theophylline alone (open circles, n = 6) and infusion of K201 in addition to theophylline (solid circles, n = 6) in the magnesium-deficient rats. The serial changes in HR, LVEDP and peak +dP/dt were similar. However, the changes in LVSP and peak -dP/dt were significantly attenuated when K201 was simultaneously added, compared with the infusion of theophylline alone. The double product increased time-dependently when K201 was added, while it did not change after infusion of theophylline alone.

DISCUSSION

In the present study, we demonstrated that theophylline infusion induced a persistent increase in heart rate and peak $+dP/dt$, and transient increase in LVEDP, but did not affect LVSP and peak $-dP/dt$ in the control rats. In contrast, in the magnesium-deficient rats, the LVSP persistently decreased and peak $-dP/dt$ persistently increased after theophylline infusion, although the increases in heart rate, peak $+dP/dt$ and LVEDP were similar to those in the control rats. In other words, theophylline decreased LVSP and increased peak $-dP/dt$ only during a state of low magnesium. In addition, the CK level after theophylline infusion was higher in the magnesium-deficient rats compared with the control rats. Interestingly, when K201 was infused along with theophylline in the magnesium-deficient rats, both the decrease in LVSP and increase in peak $-dP/dt$ were suppressed. In addition, the CK level after the infusion of theophylline plus K201 was lower compared with the infusion of theophylline alone. These results suggest that theophylline enhances left ventricular systolic function but impairs left ventricular diastolic function when the level of magnesium is low, and that K201 improves theophylline-induced left ventricular diastolic dysfunction by maintaining left ventricular systolic function when the level of magnesium is low.

Heart failure is a major public health concern and is a leading cause of morbidity and mortality in both Asia and worldwide.^{14,15} It has recently been reported that heart failure occurs even in patients who have preserved left ventricular function (i.e., a normal left ventricular ejection fraction), and this has been classified as heart failure with preserved ejection fraction (HFPEF).¹⁶ Since HFPEF is thought to be caused mainly by left ventricular diastolic dysfunction, it has also been called diastolic heart failure.¹⁷⁻¹⁹ HFPEF is present in 40-71% of patients with total heart failure, with a similar mortality rate to systolic heart failure.^{16,20} Left ventricular diastolic function is determined by left ventricular relaxation and stiffness. Peak $-dP/dt$ represents left ventricular diastolic function during isovolumic relaxation.²¹ In the current study, theophylline infusion increased peak $-dP/dt$ (i.e., impaired left ventricular relaxation) only in the magnesium-deficient rats. This result suggests that diastolic function might be more sensitive to magnesium defi-

ency than systolic function. In addition, the difference in CK levels after theophylline infusion between the control and magnesium-deficient rats suggests that magnesium deficiency might produce myocardial injury. It has been reported that activation of the renin-angiotensin system plays a role in the mechanism underlying cardiac fibrogenesis in magnesium deficiency, as activation of the renin-angiotensin system produces cardiac hypertrophy,²² which potentially leads to left ventricular diastolic dysfunction.²³

We previously reported that norepinephrine induces left ventricular diastolic dysfunction in rats fed with a high calcium diet.²⁴ We also observed that in infarcted myocardium, diastolic dysfunction was induced by norepinephrine as well as phenylephrine (α -stimulant). We speculate that this type of diastolic dysfunction is in part caused by increased intracellular calcium concentration. Caffeine and theophylline have been shown to enhance Ca^{2+} sensitivity in the myocardium.^{25,26} In the magnesium-deficient rats in the present study, not only was the serum magnesium level lower, but the serum calcium level was higher compared with the control rats. It is known that magnesium deficiency increases serum calcium level, possibly through calcium release from bone.²⁷ Magnesium administration is known to improve isoproterenol-induced left ventricular systolic and diastolic dysfunction, which may be due in part to inhibition of myocardial Ca^{2+} overload or decreased free radical formation.²⁸

In this study, we investigated the effects of K201 on theophylline-induced changes in myocardial performance, and found that K201 improved theophylline-induced impaired left ventricular relaxation during magnesium deficiency. It has been reported that K201 inhibits catecholamine-induced myocardial damage and also stores high-energy phosphate compounds such as adenosine triphosphate, thus improving myocardial energy metabolism in ischemic-reperfused myocardium.²⁹ The 1,4-benzothiazepine derivative, K201, has been shown to have a stronger protective effect on the myocardium than the 1,5-benzothiazepine derivative, diltiazem.³⁰ K201 has also been shown to improve left ventricular diastolic dysfunction more than diltiazem in rats with myocardial infarction, and in rats given a high-calcium diet and then infused with norepinephrine.³¹ In addition, K201 has been shown to stabilize ryanodine receptors on the sarcoplasmic reticulum and inhibit Ca^{2+} release from the sar-

coplasmic reticulum.^{12,13} In our magnesium-deficient rats, the CK level was lower after the infusion of theophylline plus K201 compared to the infusion of theophylline alone, and this suggests that the K201 protects against myocardial injury. These pharmacological actions of K201 could account for our findings that K201 improved theophylline-induced left ventricular diastolic dysfunction during magnesium deficiency.

Clinical implications

A low magnesium state is associated with the risk of cardiovascular diseases such as fatal arrhythmia, coronary artery disease and heart failure. Nevertheless, few studies have investigated the clinical importance of magnesium deficiency, especially in the cardiovascular field. Dietary surveys of people in the United States consistently show that the intake of magnesium is lower than the recommended amount.³² As lifestyle, and especially eating habits, have become more westernized in east Asia including Japan, magnesium deficiency has become a concern. Magnesium inadequacy can still occur when the intake is above the amount required to prevent overt deficiency. People who have gastrointestinal diseases,³³ type 2 diabetes³⁴ or alcohol dependence³³ are more likely to be at risk of magnesium inadequacy because they typically consume insufficient amounts or they have medical conditions or take medications that reduce magnesium absorption from the gut or increase losses from the body. Older adults generally have a lower dietary intake of magnesium than younger adults.³⁵ In addition, latent magnesium deficiency has increasingly been reported to be a cardiovascular risk factor.³⁶

A xanthine derivative, theophylline, is widely used to treat various respiratory diseases including chronic obstructive pulmonary disease (COPD) as a bronchodilator. The number of patients with COPD continues to increase in association with the aging society. COPD not only causes right-sided heart failure, but is also often complicated with left-sided heart failure, especially HEFPEF.³⁷ Theophylline has an apparent positive inotropic action, and thus, has long been believed to be effective to improve left-sided as well as right-sided heart failure in COPD patients.³⁸ However, our results showed that theophylline impaired left ventricular diastolic function during magnesium deficiency, although it enhanced left ventricular systolic function. These results suggest

that careful consideration is needed when prescribing theophylline for elderly patients in whom latent magnesium deficiency is possible. For such patients, cardioprotective agents rather than cardiostimulant agents may be preferable. In another result of our study, K201 improved theophylline-induced left ventricular diastolic dysfunction during magnesium deficiency without suppressing systolic function, suggesting that it may be a promising cardioprotective agent for elderly COPD patients who may be at risk of latent magnesium deficiency.

Study limitations

This study has several potential limitations. The biggest limitation is that we used only $+dP/dt$ and $-dP/dt$ as representatives of left ventricular systolic and diastolic functions, respectively. We should have assessed left ventricular function in a more comprehensive manner. End-systolic elastance, which is measured from pressure-volume loops, represents myocardial contractility, independently of both preload and afterload, and thus, is considered to be the best index for left ventricular systolic function. Although $-dP/dt$, which we used in this study, represents left ventricular diastolic function during isovolumic relaxation, the direct index of isovolumic relaxation is the time constant of the exponential left ventricular pressure regression (Tau). We should have also measured these indices to assess precise left ventricular systolic and diastolic functions. Although we did not investigate changes in left ventricular pressure-volume loop after theophylline and additional K201 infusions in the magnesium-deficient rats, estimates from our results are illustrated in Figure 4. We should also have measured echocardiographic parameters for left ventricular diastolic and systolic functions, volumes and wall thickness, because these parameters are available for clinical assessment. Although we chose the model of magnesium deficiency-induced cardiac dysfunction in this study, similar investigations using other heart failure models are needed. We used serum CK level alone as a marker of myocardial injury in this study. However, we should have measured other myocardial injury markers such as cardiac troponins because CK is not specific for the myocardium. Regarding serum biomarkers, we should also have measured plasma levels of brain natriuretic peptide or pro-brain natriuretic peptide because they are essential markers to assess cardiac function. Finally, we

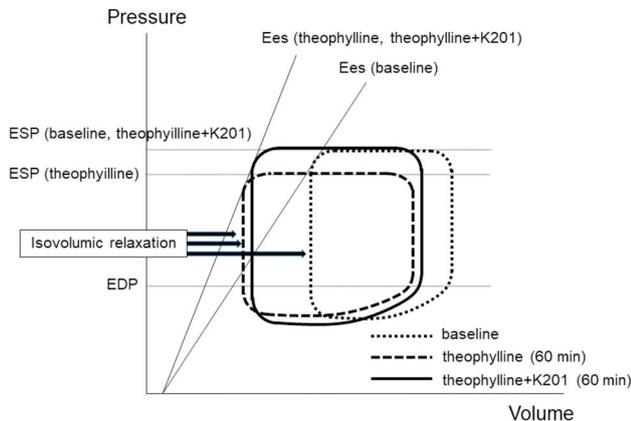


Figure 4. Change in left ventricular pressure-volume loop during magnesium deficiency, estimated from our results. In magnesium deficient rats, theophylline infusion for 15 min decreased LVSP, enhanced left ventricular systolic function, i.e., slope of end-systolic pressure-volume line (Ees) enlarged, but impairs isovolumic relaxation at 60 min after initiation of the infusion. Consequently, the pressure-volume loop might move to bottom left, but LVEDP did not change. Additional K201 infusion increased LVSP, maintained left ventricular systolic function, and improved isovolumic relaxation at 60 min after initiation of the infusion. Consequently, the pressure-volume loop might move to upper right for slope of end-systolic pressure-volume line (Ees) to be maintained from the position of theophylline alone, and LVEDP did not change.

should have measured local magnesium concentration in the myocardium in addition to serum magnesium concentration.

CONCLUSIONS

In this study, we demonstrated that theophylline impaired left ventricular diastolic function during magnesium deficiency. In addition, a 1,4-benzothiazepine derivative, K201, improved theophylline-induced left ventricular diastolic dysfunction during magnesium deficiency. K201 may provide new insights into future strategies for heart failure treatment.

CONFLICT OF INTEREST

None declared.

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