Effect of Metformin and Enalapril on the Rat' Heart with Chronic Myocardial Infarction

ผลของเมทฟอร์มินและอีนาลาพิลต่อหัวใจหนูที่มีภาวะกล้ามเนื้อหัวใจตายเรื้อรัง

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ABSTRACT

The common complications of myocardial infarction (MI) are left ventricular dysfunction and heart failure (HF) as well as sudden cardiac death. It has been known that enalapril, angiotensin converting enzyme inhibitor which is a first-line drug for patients with HF, could reduce the risk of HF progression. Recently, metformin has been demonstrated to reduce the risk for development of HF and improve survival rate in these patients. Despite a cardioprotective effect of metformin having been demonstrated, comparing data between metformin and enalapril treatment in the setting of post-MI not been well studied. This study aimed to determine the effect of metformin on cardiac function determined by heart rate variability and fractional shortening in 8-week post-MI rats, comparing with enalapril. MI rats had a significant decreased fractional shortening and an abnormal cardiac sympathovagal balance indicated by an increased ratio of low frequency to high frequency (LF/HF ratio). Enalapril significantly improved fractional shortening and attenuated cardiac sympathovagal imbalance. However, metformin did not significantly affect these parameters. In conclusion, enalapril can improve cardiac function and attenuate cardiac sympathovagal imbalance in chronic myocardial infarction rats.

บทคัดย่อ

ภาวะหัวใจล้มเหลวเป็นภาวะแทรกซ้อนภายหลังจากกล้ามเนื้อหัวใจขาดเลือด ซึ่งเป็นสาเหตุทำให้ให้ผู้ป่วยเสียชีวิต เป็นที่ทราบกันดีว่า ยาอีนาลาพิล เป็นยารักษากล้ามเนื้อหัวใจขาดเลือด ช่วยชะลอการเกิดภาวะหัวใจล้มเหลว นอกจากนี้การศึกษาทางคลินิกปัจจุบันพบว่า ยาเมทฟอร์มินช่วยลดความเสี่ยงในการเกิดภาวะหัวใจล้มเหลวและเพิ่มอัตราการรอดชีวิตในผู้ป่วยที่มีภาวะกล้ามเนื้อหัวใจขาดเลือด แม้ว่าจะมีการรายงานถึงผลของยาเมทฟอร์มิน แต่ยังไม่มีการศึกษาผลของยาเมทฟอร์มินเปรียบเทียบกับยาอีนาลาพิลในหนูที่มีภาวะกล้ามเนื้อหัวใจตายเรื้อรัง ดังนั้นการศึกษานี้จึงทำการศึกษาผลของยาเมทฟอร์มินและอีนาลาพิล ต่อการเปลี่ยนแปลงค่าความผันแปรของการเต้นของหัวใจและ %fractional shortening เปรียบเทียบกับหนูได้รับยาอีนาลาพิลเพียงอย่างเดียว จากการศึกษาพบว่า ในหนูที่ภาวะกล้ามเนื้อหัวใจตายเรื้อรังเป็นระยะเวลา 8 สัปดาห์ พบว่ามีอัตราส่วนของ high frequency และ low frequency เพิ่มขึ้นและค่า %fractional shortening ลดลง โดยพบว่ายาอีนาลาพิลสามารถลดอัตราส่วนของ LF/HF และเพิ่ม %fractional shortening ส่วนเมทฟอร์มินพบว่าไม่มีการเปลี่ยนแปลงตัวแปรเหล่านี้อย่างมีนัยสำคัญทางสถิติ

Key Words: Metformin, Enalapril, Chronic myocardial infarction

คำสำคัญ: เมทฟอร์มิน อีนาลาพิล กล้ามเนื้อหัวใจตายเรื้อรัง

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Introduction

The most common complications of acute myocardial infarction (AMI) are left ventricular (LV) dysfunction and heart failure (HF)(Sutton and Sharpe, 2000). HF is a condition in which the heart is unable to maintain appropriate cardiac output for organ supply (impaired systolic function) and unable to maintain normal ventricular filling pressure (impaired diastolic function). Enalapril is an angiotensin converting enzyme (ACE) inhibitor which has been used for the treatment of hypertension and heart failure. ACE inhibitor is a first-line drug for patients with heart failure and has been demonstrated to decrease post-MI mortality and morbidity (Jugdutt, 1995). Enalapril decreases circulating aldosterone levels, leading to decreased salt and water absorption, and a reduction in venous return to the heart (preload). Moreover, enalapril inhibits degradation of bradykinin, an arterial and venous dilation substance, thereby resulting in decreased peripheral vascular resistance (afterload) and reduced LV filling pressure (preload). Such reduction in afterload and preload prevents progressive heart failure. Previous study showed that myocardial infarction patients had a higher LF/HF ratio (Ren et al., 2011).

Metformin, a commonly used anti-diabetic drug, decreases blood glucose level by suppressing hepatic glucose production, increasing glucose uptake, and improving insulin sensitivity. The mechanism of action of metformin on the heart is proposed to be through an activation of cardiac 5' adenosine monophosphate-activated protein kinase (AMPK) (Gundewar et al., 2009a; Zhou et al., 2001). AMPK is a kinase that plays a role in the regulation of cardiac metabolism and is important for providing adequate energy supply for the heart (Gundewar et al., 2009). Metformin is cardioprotective because it can activate the AMPK and create a large translocation of glucose transporter type 4 (GLUT4) to the plasma membrane (Fu et al., 2011), therefore enabling glucose uptake into cardiomyocytes. Clinical evidence shows that metformin can reduce the risk of incident HF (Messaoudi et al., 2013) and improve survival rates in patients with HF (Papanas et al., 2012). Moreover, an animal study demonstrated that chronic metformin treatment is associated with a decreased extent of myocardial damage and an improvement of cardiac function in non-diabetic rats with myocardial infarction (Yin et al., 2011a). Despite these beneficial effects of metformin, comparing data between metformin and enalapril treatment in chronic myocardial infarction rats induced by left anterior descending coronary artery ligation has never been reported. Moreover, data regarding the effects of metformin and enalapril on the cardiac sympathovagal balance in MI model is limited. This study aimed to determine the effects of metformin on the heart rate variability and % fractional shortening in post chronic-MI rats, comparing with enalapril alone. We hypothesized that enalapril and metformin may attenuate cardiac sympathovagal imbalance and cardiac dysfunction in chronic myocardial infarction rats induced by left anterior descending coronary ligation.

Objectives of the study

This study aimed to determine the effects of metformin and enalapril on heart rate variability and %fractional shortening in chronic-MI rats induced by left anterior descending coronary artery (LAD) occlusion.

Methodology

Animals and drug administration

All experiments were conducted in accordance with an approved protocol from the Faculty of Medicine, Chiang Mai University Institutional Animal Care and Use Committee, in compliance with NIH guidelines. Rats were fed by standard laboratory chow which has energy content of 4.02 kcal/g and 19.77% total energy (%E) from fat for 12 weeks (Mouse Feed Food No. 082, C.P. Company, Bangkok, Thailand). The present study used male Wistar rats (body weight 400-450 g, n= 12) from the National animal center, Salaya campus, Mahidol University, Bangkok. Animals were acclimatized for1 week, then divided into 3 groups (n = 4/group).All animals were maintained under an environmentally controlled conditions (25 ± 0.5 °C, 12 h light/12 h dark cycle).Rats in each group were fed by gavage with vehicle (normal saline (NSS)): 2 ml/kg body weight (BW)/day), enalapril (*Berlin Pharmaceutical Industry*, Thailand: 10 mg/kg BW, once a day) and metformin (Glucophage, Merck Serono, Thailand: 15 mg/kg BW, b.i.d) for 8 weeks after LAD ligation.

Myocardial infarction induction procedure

Myocardial infarction was induced by ligation of the LAD, as previously described (Lekawanvijit et al., 2012). Briefly, animals were incubated and mechanically ventilated with 2 % isoflurane with 1.0 L/min oxygen. After left sided thoracotomy, MI was induced by ligating LAD 4 mm from its origin and tied securely. Subsequently, positive pressure was applied to the lungs via the ventilator before closing the chest wall and incision. Animals were maintained during a period of 8 weeks to allow left ventricular infarction area to evolve into a fully healed scar. Complete occlusion of left coronary artery in rats produced transmural infarct at apex and anterior free wall of left ventricle were included in this study (Goldman and Raya, 1995).

Heart rate variability (HRV) determination

Prior to and at 8 weeks after LAD ligation, rats were anesthetized with isoflurane (FORANE, Abbott Laboratories, Kent, England) for electrocardiogram (ECG) limb lead insertion. After gaining full conscious, ECG was recorded in each rat using Chart 5.0 program for 20 mins. Heart rate variability (HRV) was analyzed using MATLAB program (Perakakis et al., 2010). Frequency domain consisting of high-frequency (HF) band and low-frequency (LF) band were analyzed for cardiac sympathovagal imbalance (Fioranelli et al., 1999). LF/HF ratio was used to indicate cardiac sympathovagal activity (Incharoen et al., 2007).

Echocardiogram

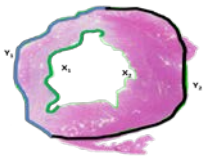
Left ventricular function was assessed by echocardiography. Echocardiographic measurement was performed under general anaesthesia (2% isoflurane). Both 2-dimentional (2D) images in parasternal long axis and short axis view and 2-D guided M-mode tracing was obtained. Short axis views were recorded at the level of mid-papillary muscles. LV fractional shortening (%FS) was calculated using the following equation:

$$FS\ (\%) = [(LVIDd - LVIDs)/LVIDd] \times 100$$

At the end of the echocardiography protocol, the animal was placed to recover in a recovery box until gaining full consciousness.

Myocardial infarct size assessment

The heart was excised and rinsed with physiological saline. The left ventricle was divided horizontally into 3 sections. The middle section was fixed in 10% buffered formalin solution for histology. Mid-LV cross sections were stained with Hematoxylin and Eosin (H&E) and scanned for infarct size analysis. Assessment was performed in animals with transmural infarction where the full thickness of LV wall from endocardial to epicardial was infarcted. Infarct size was reported as the average of the proportions of LV endocardial and epicardial circumferences occupied by the infarct (Figure 2) as previously described (Steven Goldman and Raya, 1995). Infarct size was calculated as the following;



$$\text{Infarct size (\%)} = [X_1/(X_1+X_2) + Y_1/(Y_1+Y_2)]/2 * 100$$

Where;

X_1 = endocardial length of infarct (green line)

X_1+X_2 = inner circumference of left ventricle

Y_1 = epicardial length of infarct (blue line)

Y_1+Y_2 = outer circumference of left ventricle

Data analysis

All data were expressed as mean ± SE. One-way ANOVA followed by LSD post-hoc test was used to determine a difference between groups. $P<0.05$ was considered statistically significant.

Results

Heart rate variability (HRV)

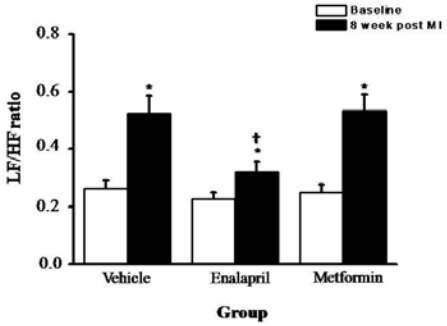


Figure 1 LF/HF ratio of myocardial infarction rats treated with vehicle, Enalapril, Metformin (n=4 in each group). *$P<0.05$ vs. baseline, †$P<0.05$ vs Vehicle group.

At 8 weeks post-MI, LF/HF ratio was significantly increased ($p<0.05$), compared with baseline. LF/HF ratio was reduced significantly in MI animals treated with enalapril but not in metformin-treated group when compared with the vehicle-treated group. (Figure 1)

Echocardiographic parameter

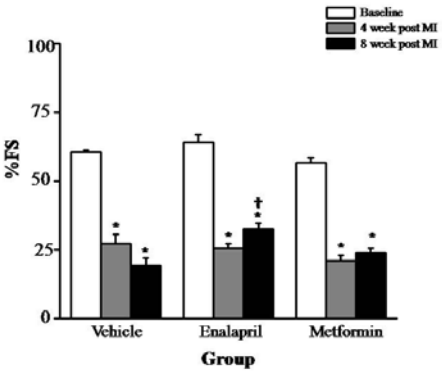


Figure 2 Fractional shortening of MI rats treated with vehicle, Enalapril, Metformin (n=4 in each

group). *P<0.05 vs. baseline, †P<0.05 vs Vehicle group.

Eight weeks after MI, there was a significant improvement of the %FS in enalapril- treated group but not in metformin-treated group when compared with the vehicle-treated group (Figure 2).

Myocardial infarct size

LAD ligation resulted in infarct size of 45 ± 2%, 41± 4% and 31± 6% in vehicle group, enalapril-treated group and metformin-treated group, respectively. There was no significant difference in the infarct size among groups.

Discussion and Conclusions

The present study demonstrated that LAD ligation developed myocardial infarction characterized by decreased fractional shortening and is associated with depressed cardiac sympathovagal balance as indicated by an increased LF/HF ratio. Enalapril, but not metformin, significantly improved LF/HF ratio and fractional shortening. There is no difference in infarct size among the three MI groups.

Heart rate variability (HRV) is an indicator of cardiac sympathovagal imbalance. (Handa et al., 2012) Our study demonstrated that MI rats had a decreased fractional shortening in associated with an increased LF/HF ratio. (Zanobetti et al., 2010) The finding was consistent with previous studies that HRV is reduced in myocardial infarction. The mechanism responsible for an improvement of cardiac sympathovagal imbalance by enalapril is likely mediated by increasing parasympathetic activity and decreasing sympathetic activity. This finding is consistent with previous clinical studies reporting that Enalapril treatment could improve cardiac sympathovagal balance in patient with congestive heart failure (Zhang et al., 1995;Sturm et al., 2000).

Previous studies demonstrated that enalapril attenuated the increased fractional shortening in cardiomyopathy rats (Hiona et al., 2011) and in patients with heart failure. (Yoshimura et al., 2002) Reducing aldosterone levels by ACE inhibitors leads to decreased salt and water reabsorption and a preload reduction. Moreover, Enalapril inhibits degradation of bradykinin, an arterial and venous dilation substance, thereby resulting in decreased peripheral vascular resistance (afterload) and reduced LV filling pressure (preload). Such reductions in afterload and preload lead to improved fractional shortening and prevent progression of HF. Some studies reported that metformin significantly improve fractional shortening after myocardial infarction (Gundewar et al., 2009; Yin et al., 2011) whilst the present study only observed a trend of improvement.

In conclusion, we demonstrated that MI rats induced by LAD ligation developed a depressed HRV, and cardiac contractile dysfunction. Enalapril attenuated these impairments by improving HRV and fractional shortening. However, metformin did not significantly affect these parameters.

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