Effects of simvastatin on ion channel currents in ventricular myocytes from rabbit with acute myocardial infarction

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Objective To investigate the effects of simvastatin on membrane ionic currents in left ventricular myocytes after acute myocardial infarction (AMI), so as to explore the ionic mechanism of statin treatment for antiarrhythmia. Methods Forty-five New Zealand rabbits were randomly divided into three groups: AMI group, simvastatin intervention group (statin group) and sham-operated control group (CON). Rabbits were infarcted by ligation of the left anterior descending coronary artery after administration of oral simvastatin 5 mg kg⁻¹ d⁻¹ (Statin group) or placebo (AMI group) for 3 days. Twenty-four hours later, single ventricular myocytes were isolated enzymatically from the epicardial zone of the infarcted region. Whole cell patch clamp technique was used to record membrane ionic currents, including sodium current (I Na ), L-type calcium current (I Ca,L ) and transient outward potassium current (I K ). Results ① There was no significant difference in serum cholesterol concentration among three groups. ② The peak I Na current density (at -30 mV) was significantly decreased in AMI group (-23.26±5.18) compared with CON (-42.78±5.48, P<0.05), while it was significantly increased in Statin group (39.23±5.45) compared with AMI group (P<0.01). The peak I Ca,L current density (at 0 mV) was significantly decreased in AMI group (-3.23±0.91) compared with CON (-4.56±1.01, P<0.05), while it was significantly increased in Statin group (-4.18±0.95) compared with AMI group (P<0.05). The I K current density (at +60 mV) was significantly decreased in AMI group (10.41±1.93) compared with CON (17.4±3.13, P<0.01), while it was significantly increased in Statin group (16.21±2.42) compared with AMI group (P<0.01). Conclusions AMI induces significant down-regulation of I Na , I Ca,L and I K . Pretreatment with simvastatin could attenuate this change without lowering the serum cholesterol level, suggesting that simvastatin reverse this electrical remodeling, thus contributing to the ionic mechanism of statin treatment for antiarrhythmia. (J Geriatr Cardiol 2008; 5:179-181)

Key words simvastatin; myocardial infarction; ionic channels; patch-clamp; rabbit

Acute myocardial infarction (AMI) is frequently associated with ventricular tachyarrhythmias, including ventricular tachycardia (VT) and ventricular fibrillation (VF), which are the major direct causes of sudden cardiac death in patients with coronary artery disease. After coronary artery occlusion, surviving myocardium in and around the infarct zone plays an important role in arrhythmogenesis, which is thought to be strongly associated with the alterations of electrophysiological characteristics, known as “electrical remodeling”. ① It has been shown that pretreatment with statin is effective in preventing arrhythmia after AMI in experimental models and clinical study. ②,③ but its electrophysiological mechanism is unclear. The present study was designed to examine the effect of pretreatment with simvastatin on the changes in membrane ionic currents, including sodium channel current (I Na ), L-type calcium channel current (I Ca,L ) and transient outward potassium channel current (I K ) in left ventricular myocytes of normcholesteolemic rabbits undergoing AMI, so as to explore the ionic mechanism responsible for the anti-arrhythmic effect of statin.

Materials and methods

Materials Adult healthy New Zealand rabbits of either sex weighing 2.0-2.5 kg were provided by the Experimental Animal Center of Hebei Medical University, China. Forty-five rabbits were randomly divided into three groups: AMI group, simvastatin intervention group (Statin group) and sham-operated control group (CON). The main drugs include: HEPES, EGTA, 4-aminopyridine (4-AP), collagenase type I, BSA and choline chloride, were purchased from Sigma (USA); the others were domestic products of analytical grade. Simvastatin was provided by Merck Sharp & Dohme Corporation.

Cholesterol measurement Before the surgical procedures, the serum cholesterol was measured in all three groups.
Surgical procedures

After consecutive administration of oral simvastatin (5 mg kg⁻¹ d⁻¹) (Statin group) or placebo (AMI group and CON) for 3 days, rabbits were infarcted by ligation of the left anterior descending coronary artery. In the sham-operated control group, to rule out possible changes in myocyte electrophysiology that resulted from the surgical procedure, rabbits were subjected to the standard surgical procedures without coronary ligation.

Isolation of ventricular myocytes and measurement of current

24 hours later, single rabbit ventricular myocytes were isolated enzymatically (0.04% collagenase type I, sigma) as previously described[4], myocytes were obtained from the epicardial zone of the infracted area (AMI and Statin group), and the same anatomy region of control noninfarcted hearts (CON).

Electrophysiological recording

Transmembrane currents were recorded using the whole cell patch-clamp techniques similar to that previously described. I_{Na}, I_{Ca-L}, I_{to} were recorded with an EPC-9 patch clamp amplifier (Germany, HEKA). Data acquisition and processing were performed by the pulse+ pulsefit software (HEKA, version 8.53). Current data could then be expressed as current density (pA/pF) by normalized each current value by the cell’s capacitive value.

Results

The concentration of serum cholesterol

The serum cholesterol concentration (mmol/L) in CON, AMI, and Statin groups was 1.79±0.58 (n=15), 1.99±0.21 (n=15), 1.90±0.28 (n=15), respectively. There were no significant differences in serum cholesterol concentration among three groups (P>0.05).

Effect of simvastatin on I_{Na} in rabbit myocytes

The mean current density - voltage relationships curve (I-V curve) for I_{Na} was illustrated in Fig.1. The peak I_{Na} current density (at −30 mV) in CON, AMI group and Statin group were −42.78±5.48 (n=16), −23.26±5.18 (n=12), −39.23±5.45 pA/pF (n=13), respectively (AMI group vs CON and Statin group, P<0.01). The peak I_{Na} current density was down-regulated in ventricular myocytes from AMI group compared with CON, simvastatin could ameliorate this change. This was not accompanied by a shift in the I_{Na} current density - voltage relation.

Effect of simvastatin on I_{Ca-L} in rabbit myocytes

The mean current density - voltage relationships curve for I_{Ca-L} was illustrated in Fig.2. The peak The peak I_{Ca-L} current density (at 0 mV) was significantly decreased in AMI group (−4.41±0.93 pA/pF, n=12) compared with CON (−4.56±0.91 pA/pF, n=15), (P<0.01), while it was significantly increased in Statin group (16.21±2.42 pA/pF, n=13) compared with AMI group (P<0.01).

Effect of simvastatin on I_{to} in rabbit myocytes

The mean current density - voltage relationships curve for I_{to} was illustrated in Fig.3. The I_{to} current density (at +60 mV) was significantly decreased in AMI group (−4.18±0.95 pA/pF, n=12) compared with AMI group (P<0.05). The I_{to} current density was significantly increased in Statin group (−4.18±0.95 pA/pF, n=12) compared with AMI group (P<0.05).

Discussion

Hydroxymethylglutary coenzyme A reductase inhibitors (statins) have been shown to have effects independent of their cholesterol-lowering effects, referred to as pleiotropic effects. Fonarow et al. showed that new or continued treatment with a statin in the first 24 hours of AMI patients was associated with a decreased risk of mortality compared with no statin use (4.0% and 5.3% compared with 15.4% no statin), and early statin use was associated with a lower incidence of cardiogenic shock, arrhythmias, cardiac arrest and rupture. This study indicates that statin may contrib-
In conclusion, our study showed that AMI induces significant down-regulation of \( I_{\text{Na}} \), \( I_{\text{Ca-L}} \), and \( I_{\text{K}} \), and pretreatment with simvastatin could ameliorate this change in transmembrane ion channel properties. So, we speculate that statin may exert its anti-arrhythmic effect through the whole cardioprotective pleiotropic effects.

In conclusion, our study showed that AMI induces significant down-regulation of \( I_{\text{Na}} \), \( I_{\text{Ca-L}} \), and \( I_{\text{K}} \), and pretreatment with simvastatin could attenuate these changes without lowering the serum cholesterol level, suggesting that simvastatin could reverse this electrical remodeling, thus contributing to the ionic mechanism responsible for the anti-arrhythmic effect of statin. It might imply that the ionic mechanism of statin for anti-arrhythmia is a pharmaceutical effect independent of decreasing cholesterol. Accordingly, simvastatin, through the anti-arrhythmic effects, may contribute to reducing cardiovascular mortality. So preventing or reversing electrical remodeling induced by ischemia and reperfusion injury should also be a clinical therapeutic target. Our findings expand the pleiotropic spectrum of the statins’ favorable effects on cardiovascular diseases.

References

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