Clinical Research

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 of rabbit heart suffering from 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. 24 h 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 (Ito).

Results There was not significant difference in serum cholesterol concentration among three groups.

The peak INa current density (at –30 mV) was significantly decreased in AMI group (–23.26±5.18, n=12), compared with CON (–42.78±5.48, n=16), P<0.05, while it was significantly increased in Statin group (–39.23±5.45 pA/pF, n=13) compared with AMI group, P<0.01. The peak Ica-L current density (at 0 mV) was significantly decreased in AMI group (–3.23±0.91 pA/pF, n=12) compared with CON (–4.56±1.01 pA/pF, n=15), P<0.05, while it was significantly increased in Statin group (–4.18±0.95 pA/pF, n=12) compared with AMI group, P<0.05.

The Ito current density (at +60 mV) was significantly decreased in AMI group (10.41±1.93 pA/pF, n=12) compared with CON (17.41±3.13 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.

Conclusions AMI induces significant down-regulation of INa, Ica-L, and Ito; pretreatment with simvastatin could attenuate this change without lowering the serum cholesterol level, suggesting that simvastatin could reverse this electrical remodeling, thus contributing to the ionic mechanism of statin treatment for antiarrhythmia.

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

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Materials and Methods

Materials Adult healthy New Zeland 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 described⁴, 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).

Electrophysiologic recording
Transmembrane currents were recorded using the whole cell patch-clamp techniques similar to that previously described⁵. 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 of unremarkable 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 INa 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 current density was significantly decreased in AMI 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 effects⁶. 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). Early statin use was associated with a lower incidence of cardiogenic shock, arrhythmias, cardiac arrest and rupture⁷. Therefore, this indicates that statin may contribute to its cardioprotective effects partly through its anti-
AMI, which was due to INa attenuation by pretreatment of simvastatin for 3 days before infarcted heart, thus contributing to various ventricular myocytes in and around the infarct zone of the abnormal transmembrane action potentials of the surviving may underlie the altered electrophysiological activity and vulnerability plaque, amelioration of endothelial dysfunction, reversing electrical remodeling, which may be partly responsible for its anti-arrhythmic effect of statin. It might imply that the ionic mechanism of statin from anti-arrhythmia is a pharmaceutical effect independent on 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 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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