Effects of atorvastatin on time-dependent change of fast sodium current in simulated acute ischaemic ventricular myocytes

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Abstract

Introduction: Our previous experiments showed that the transient sodium current (I_{Na}) was abnormally increased in early ischaemia and atorvastatin could inhibit I_{Na}. The aim of this study was to observe the time-dependent effects of simulated ischaemia on I_{Na} and characterise the direct effects of atorvastatin on ischaemic I_{Na}.

Methods: Left ventricular myocytes were isolated from Wistar rats and randomly divided into two groups: a control group (normal to simulated ischaemia) and a statin group (normal to simulated ischaemia with 5 μmol/l atorvastatin). The I_{Na} was recorded under normal conditions (as baseline) by whole-cell patch clamp and recorded from three to 21 minutes in the next phase of simulated ischaemic conditions.

Results: In the control group, normalised I_{Na} (at –40 mV) was increased to the peak (1.15 ± 0.08 mA) at three minutes of ischaemia compared with baseline (0.95 ± 0.04 mA, $p < 0.01$), it subsequently returned to baseline levels at nine and 11 minutes of ischaemia (0.98 ± 0.12 and 0.92 ± 0.12 mA, respectively), and persistently decreased with prolonged ischaemic time. In the statin group, there were no differences between baseline and the early stages of ischaemia (0.97 ± 0.04 mA at baseline vs 0.92 ± 0.12 mA in ischaemia for three minutes, $p > 0.05$).

Conclusion: Our results suggest that, in the early stages of ischaemia, changes in I_{Na} in ventricular myocytes are time-dependent, showing an initial increase followed by a decrease, while atorvastatin inhibited the transient increase in I_{Na} and made the change more gradual.

Keywords: ventricular myocytes, sodium, ventricular arrhythmia, membrane potential, statin

Submitted 11/7/18, accepted 25/4/19
Published online 28/7/19
Cardiovasc J Afr 2019; **30**: 268–274 www.cvja.co.za

DOI: 10.5830/CVJA-2019-021

Clinically, acute ischaemia is one of the common causes of malignant ventricular arrhythmias.[1] A retrospective study showed that 7.5% of patients with acute myocardial infarction developed ventricular arrhythmias, most of which (78%) occurred within the first 48 hours of ischaemic symptoms,[2] suggesting that electrical activities are very unstable in the early stage of ventricular ischaemia.

Sodium current (I_{Na}) is the starting current of the action potential and affects the shape and conduction of the action potential.[3] It is one of the most common targets to cause and treat arrhythmias. Animal experiments found that in an aconitine-induced arrhythmia model,[4] increased I_{Na} could lead to pre-contraction and even ventricular arrhythmias. Therefore I_{Na} plays an important role in arrhythmogenesis.

Previous studies have shown that I_{Na} would be decreased or Nav1.5, which is the ion channel protein of I_{Na}, would be downregulated in the ischaemic condition.[5,6] However in our pre-experiment of simulated ischaemia, peak I_{Na} was transiently increased in the very early stage of ischaemia (three to five minutes), suggesting unstable early ischaemic electrical activity. As the decreased I_{Na} demonstrated in ischaemia or simulated ischaemia usually needs myocyte exposure for more than 10 minutes,[5] this indicates that time is a key factor affecting I_{Na} in the ischaemic state.

On the other hand, as the basic therapeutic agents of acute coronary syndrome, statins may reduce the incidence of ischaemic ventricular arrhythmias[7,8] and can prevent sudden cardiac death,[9] as well as other cardiovascular events. However, the mechanisms are controversial. One view is that electrical protection from the statin is secondary to a decrease in low-density lipoprotein cholesterol, whereas another view is that statins act as an upstream protection on the basis of pleiotropic effects.[10] In addition, Vaquero *et al.*[11] confirmed that atorvastatin and simvastatin had an inhibitory effect on atrial plateau currents [hKv1.5 and Kv4.3 channels, while $I_{Ca,L}$ (L-type calcium current) could also be blocked by simvastatin acid] at the cellular level. Similarly, there is a direct electrical effect on the I_{Na} of ventricular myocytes in the early stage of ischaemia only.

We assumed that the effect of ischaemia on I_{Na} was time-dependent, that I_{Na} may be transiently increased during the first 10 minutes of ischaemia, and that atorvastatin could inhibit this phenomenon. Therefore we used a patch-clamp technique to observe the time-dependent effects of simulated ischaemia on I_{Na} in ventricular myocytes by setting the observation interval to two minutes. In addition, we also applied atorvastatin on the above basis, in order to observe its direct effect on I_{Na} in the early ischaemic condition.

Methods

Thirty Wistar rats (300 ± 50 g, male and female) were purchased from the Chinese Academy of Medical Sciences Institute of

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Radiation Medicine Experimental Animal Centre. All study protocols and use of rats were approved by the Institutional Animal Care and Use Committee of Tianjin Medical University (Tianjin, China).

Ca^{2+}-free Tyrode solution contained (mM): NaCl 137, KCl 5.4, $MgCl_2$ 1, NaH_2PO_4 0.33, HEPES 10, and glucose 10 (pH 7.4 with NaOH). KB solution contained (mM): L-glutamic acid 50, KCl 40, $MgCl_2$ 3, KH_2PO_4 20, taurine 20, KOH 70, EGTA 0.5, HEPES 10, and glucose 10 (pH 7.4 with KOH). The pipette solution contained (mM): CsCl 140, NaCl 10, EGTA 5, HEPES 5, Na_2ATP_5 (pH 7.3 with CsOH). The normal extracellular solution contained (mM): choline-Cl 120, NaCl 25, CsOH 4, $CaCl_2$ 0.1, $CoCl_2$ 2, $MgCl_2$ 1, HEPES 10, and glucose 10 (pH 7.4 with CsOH). The simulated ischaemic extracellular solution contained (mM): choline-Cl 120, NaCl 25, CsOH 4, $CaCl_2$ 0.1, $CoCl_2$ 2, $MgCl_2$ 1, HEPES 10, and natrium lacticum 20 (adjusted to pH 6.8 and filled with nitrogen for more than five minutes before using). Atorvastatin calcium (USP Corporation, Lot 344423-98-9) was dissolved in the ischaemic extracellular solution to prepare the drug solution containing 5 μM atorvastatin (usually 3.02 mg of atorvastatin calcium was dissolved in 500 ml of extracellular solution).

For isolation of the myocytes, single ventricular myocytes were dissociated from hearts of Wistar rats using type II collagenase (Gibco). Rats were weighted, heparinised (5 000 UI/kg), anaesthetised with chloral hydrate (40 mg/kg), the chest was opened and the hearts were removed, and then the rats were euthanised. The heart was immersed in Ca^{2+}-free Tyrode solution (4°C) and immediately clipped.

The heart was cannulated through the aorta and mounted on a Langendorff perfusion apparatus (100% O_2, 37°C, perfusion pressure 70 cm H_2O). It was retrogradely perfused with Ca^{2+}-free Tyrode solution until the blood was washed out, followed by perfusion with the same Ca^{2+}-free Tyrode solution supplemented with 0.6 mg/ml collagenase II and 0.5 mg/ml albumin bovine serum (68 kD, Roche). As the drip rate reached 20 ml/min and the colour of the heart changed to orange and transparent, the perfusion was complete.

The heart was then removed into KB solution (37°C). The free left ventricular wall was cut into approximately 8 × 2-mm sections with a fine scissors and the endocardium and epicardium were removed in the KB solution. The mid-myocardial section was cut up and agitated with a dropper in order to obtain isolated cells. The cell suspension was then filtered with a strainer (200 mesh). Before recording, the myocytes were placed in filtered KB solution for more than two hours.

I_{Na} was recorded at room temperature (25°C) using the whole-cell configuration of the patch-clamp with Axopatch 700B amplifiers and pClamp 10.1 software (Axon Instruments, USA). Pipettes were pulled from borosilicate capillary tubes using a programmable horizontal micro-electrode puller (P-97, Sutter Instruments, USA) and heat polished with a microforge (MF-830, Narishige). Micropipette resistance was kept at 2–5 MΩ when filled with pipette solution and immersed in the extracellular solution.

The cells were placed in normal extracellular solution for rupture of the membrane, compensation for membrane capacitance and series resistor (75%), and the currents were recorded for baseline. Then the cell bath was perfused with the simulated ischaemic solution (control group) or drug solution (statin group) for three minutes (3 ml/min). At this time, the extracellular solution was replaced completely and we considered the time after one minute of perfusion as the zero point for the start of ischaemia. The cells were then left standing for one minute to avoid interference from mechanical vibration. Thereafter I_{Na} was recorded every two minutes from three minutes after the start of ischaemia to 21 minutes, in both the statin and the control groups.

The holding potential was maintained at –90 mV and the protocol for recording I_{Na} was composed of 50-ms pulses that were imposed in 5-mV increments between –80 and +50 mV, and pulse frequency was 2.5 Hz, which was matched with the rat's natural heart rate. In order to trace the inactivation curves, a double-pulse protocol was set up: the first 50-ms conditioning pulses were imposed in 5-mV increments between –80 and +50 mV, each of which was followed by a test pulse to +10 mV. Finally, to describe the recovery curves after inactivation, another double-pulse protocol was used: the first conditional pulses were imposed at –40 mV for 50 ms, each of which was followed by a fixed 80-ms test pulse from –90 to –40 mV, and the interval between the two pulses was increased in 2-ms increments from 2 to 76 ms.

Statistical analysis

In order to eliminate the effect of cell size on I_{Na}, the I_{Na} from different myocytes should be standardised. As atorvastatin may also affect the membrane capacitance, which may become a confounding factor in the current density, we used the relative current value as the normalised I_{Na} in order to evaluate the effects of atorvastatin on the peak value of the I_{Na}.

The Boltzmann equation was used to fit the activation and inactivation curves, and the recovery curve after inactivation was fitted with an exponential equation. We observed the normalised I_{Na}, membrane potential at 50% maximal activation ($V_{1/2,a}$), offsetting of the activation curve (K_a), membrane potential at 50% maximal inactivation ($V_{1/2,i}$), offsetting of the inactivation curve (K_i) and recovery constant (τ). The data were analysed by means of variance analysis of repeated measurement data, and the gating characteristics were analysed with the allogeneic paired *t*-test; $p < 0.05$ indicated that the difference was statistically significant.

Results

Effect of ischaemia on I_{Na} in the early stage after perfusion: Previous experiments showed that ischaemia suppressed the amplitude of I_{Na}, but we observed the normalised I_{Na} was transiently increased in the very early stage of ischaemia in the pre-experiment. In order to verify the increased current was not associated with the mechanical effect of perfusion, we compared the effect of ischaemic and normal extracellular solutions on I_{Na} in the same way. We found compared with normal extracellular solution, normalised I_{Na} was transiently increased after perfusion with ischaemic extracellular solution, while simulated ischaemia was for three minutes (0.92 ± 0.04 vs 1.42 ± 0.34 mA, $p < 0.01$; Fig. 1).

Effect of atorvastatin on I_{Na} in the early stage of ischaemia: When entering the simulated ischaemic state, the whole-cell currents of control and statin groups both changed over time (Fig. 2). Because of the voltage-dependent characteristics, the

maximum currents appeared at –40-mV test potential (Fig. 3), which was used to analyse the time-dependent effects of ischaemia and atorvastatin on I_{Na}.

In the control group, the normalised I_{Na} increased above baseline in the first three to seven minutes of simulated ischaemia, and peaked at three minutes (Figs 3A, 4). Compared with the three-minute point, the normalised I_{Na} decreased at seven minutes ($p = 0.0321$). At the nine- and 11-minute points, the normalised I_{Na} returned to baseline ($p = 0.3209$ and 0.5505, respectively). With the recording time extended, the normalised I_{Na} was lower than baseline ($p < 0.05$) and gradually decreased from 13 to 21 minutes (13 vs 15 minutes, $p = 0.0270$; 15 vs 17 minutes, $p = 0.0146$; 19 vs 21 minutes, $p = 0.0014$, respectively; Fig. 4).

In the statin group, the normalised I_{Na} gradually decreased during the whole time of simulated ischaemia. It decreased by 0.09 ± 0.03 mA at five minutes compared with baseline ($p = 0.0163$), decreased by 0.08 ± 0.03 mA at 13 minutes compared with five minutes ($p = 0.0256$), and continued decreasing by 0.09 ± 0.02 mA ($p = 0.0040$) at 21 minutes compared with 13 minutes (Fig. 4).

Comparing normalised I_{Na} between the two groups (Fig. 4), there were no differences at baseline and 11 to 19 minutes of ischaemia ($p > 0.05$). Normalised I_{Na} in the statin group was lower than in the control group at three to nine minutes of ischaemia ($p < 0.05$), while at 21 minutes, I_{Na} in the statin group was higher than in the control group ($p < 0.05$).

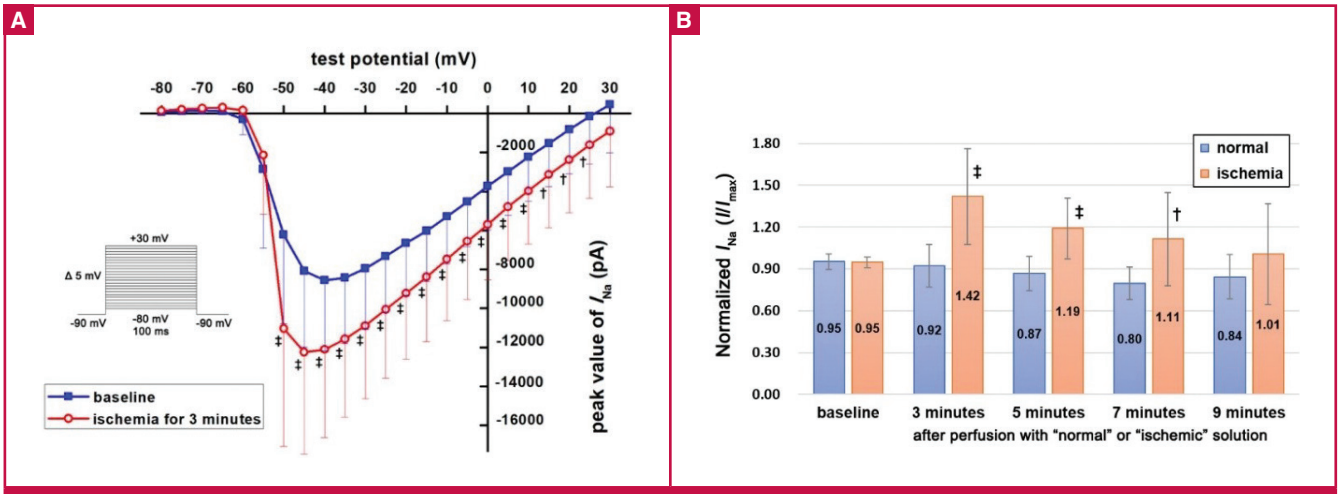

Fig. 1. Effects of ischaemia on I_{Na} in the very early stage after perfusion. (A) Current–voltage (I–V) curves between baseline and ischaemia. When ventricular myocytes were perfused with ischaemic solution, the peak value of I_{Na} was voltage-dependently increased in the stage of ischaemia after three minutes. Compared with baseline, $^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$. (B) Normalised I_{Na} after perfusion with normal and ischaemic extracellular solution. In the first 10 minutes after perfusion, normalised I_{Na} was transiently increased when perfused with ischaemic solution, whereas there was little change when perfused with normal solution, which excluded the effects of mechanical action on I_{Na}. Compared with normal solution, $^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$.

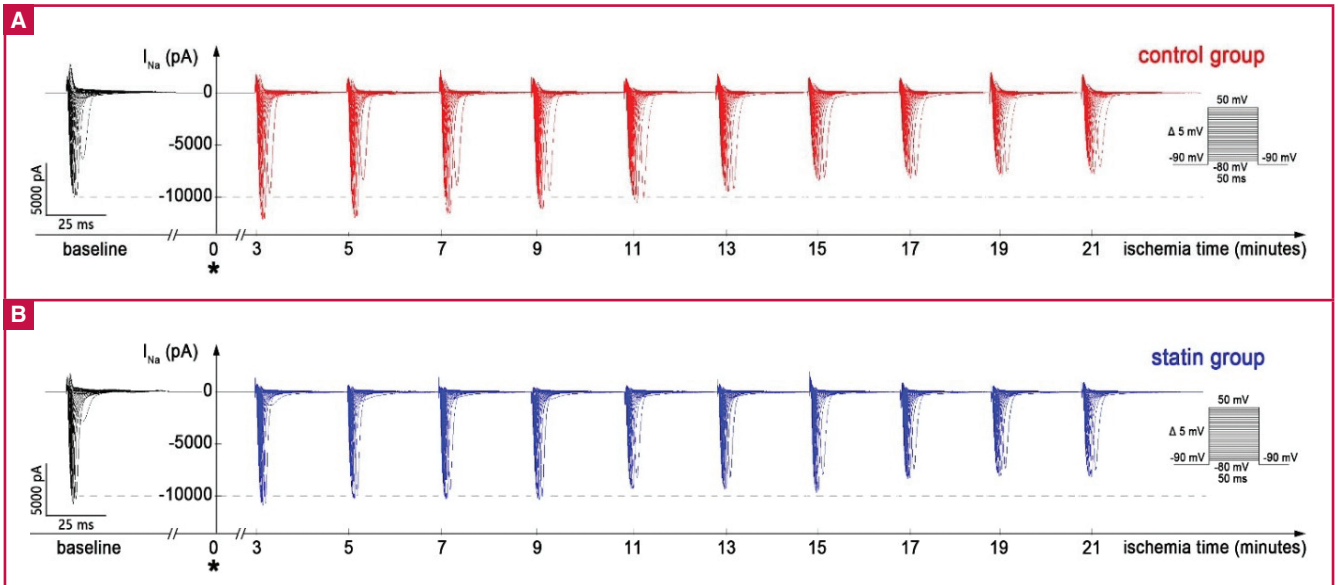

Fig. 2. Trend of whole-cell currents of I_{Na} over time. (A) Whole-cell currents in the control group. (B) Whole-cell currents in the statin group. *zero point of simulated ischaemia.

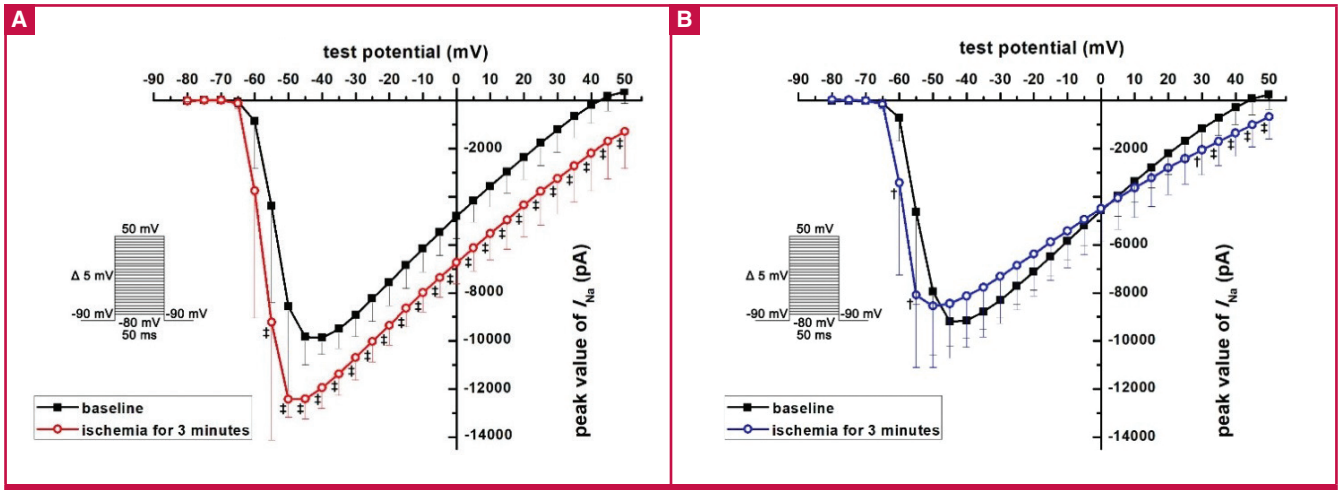

Fig. 3. Current–voltage (I–V) curves between baseline and ischaemia for three minutes. (A) I–V curve of control group, which was down-shifted in the very early stage of ischaemia, and represented the increase of I_{Na} at the test potential from –55 to 50 mV. (B) I–V curve of statin group, which was little changed in the early ischaemic condition, compared to the baseline.

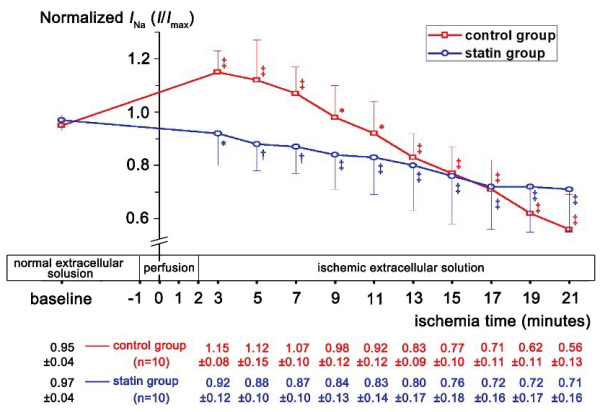

Fig. 4. Time trend of normalised I_{Na} at –40-mV test potential. When entering the simulated ischaemic stage, the normalised I_{Na} in the control group was transiently increased during the first three to seven minutes, and then attenuated rapidly, while in the statin group, the normalised I_{Na} gradually decreased during the whole of the simulated ischaemia.

Table 1. Gating characteristics at three minutes of simulated ischaemia ($\bar{x} \pm s$)

	Activation (n = 8)		*Inactivation* (n = 8)		*Resurrection* (n = 9)
	$V_{1/2,a}$ (*mV*)	K_a (*mV*)	$V_{1/2,i}$ (*mV*)	K_i (*mV*)	τ (*ms*)
Control group					
Baseline (A_1)	–54.91 ± 4.22	1.45 ± 0.48	–62.84 ± 2.50	4.52 ± 0.97	34.23 ± 4.40
Ischaemia (B_1)	–58.82 ± 3.65	0.90 ± 0.31	–65.19 ± 3.33	4.28 ± 1.11	25.54 ± 6.41
Value of B_1–A_1	–3.90 ± 2.16	–0.55 ± 0.44	–2.35 ± 1.71	–0.23 ± 0.38	–8.69 ± 4.75
p (A_1:B_1)	0.0014	0.0090	0.0061	0.1238	0.0006
Statin group					
Baseline (A_2)	–54.70 ± 3.54*	1.41 ± 0.65*	–63.33 ± 2.24*	4.92 ± 0.55*	34.58 ± 8.55*
Ischemia (B_2)	–59.16 ± 3.53	1.03 ± 0.58	–66.45 ± 1.91	4.12 ± 0.56	30.22 ± 9.65
Value of B_2–A_2	–4.47 ± 1.97	–0.38 ± 0.35	–3.12 ± 1.00	–0.81 ± 0.35†	–4.36 ± 4.82†
p (A_2:B_2)	0.0004	0.0169	0.0000	0.0004	0.0263

Compared with the baseline of the control group, *$p > 0.2$, and compared with the value of B_1–A_1, †$p < 0.05$.

Table 1 shows the gating characteristics of the two groups. Compared with baseline, in the three minutes of simulated ischaemia, the curves of activation and inactivation were shifted negatively (Fig. 5A–D), and K_a and τ were decreased in both groups (Table 1, Fig. 5A, B, E, F). At three minutes of simulated ischaemia, K_i in the statin group was lower than in the control group ($p < 0.05$), and τ in the statin group was higher than in the control group ($p < 0.05$; Table 1, Fig. 5C–F).

Discussion

Sodium current plays an important role in ischaemic ventricular arrhythmias, which may affect cardiac conductivity and irritation.[3] Previous studies have shown that sodium current may decrease in the ischaemic state,[5] but in our study, the current transiently increased in the early stage of ischaemia. Ventricular arrhythmias mainly appear in acute myocardial ischaemia in two time periods after birth (0–0.5 and 1.5–9 hours).[12]

In view of the relationship between ischaemic time and the degree of injury, we hypothesised that the change of I_{Na} in simulated ischaemia may be time-dependent. To observe the instantaneous change in I_{Na}, the measurement time interval was shortened to two minutes. The results showed that I_{Na} was transiently increased and peaked at three minutes after simulated ischaemia. At this time, the $V_{1/2,a}$ and $V_{1/2,i}$ were both decreased, which represented the activation and inactivation thresholds, respectively, and meant that both the activation and inactivation processes would be much easier at the early stage of ischaemia. In addition, decreased K_a and τ indicated that the processes of channel activation and recovery had been changed much faster (Fig. 5).

In summary, these changed gating characteristics indicated that channel transition between open and closed states became more frequent, and the open probability of sodium channels per unit time had been increased. Since $I_m = i\ P_0\ N$ (where I_m is the whole-cell current, i is the single-channel current, P_0 is the open probability, and N is the number of channels),[13] the whole-cell I_{Na} had been consequently increased at three minutes of simulated ischaemia. However, this experiment also showed

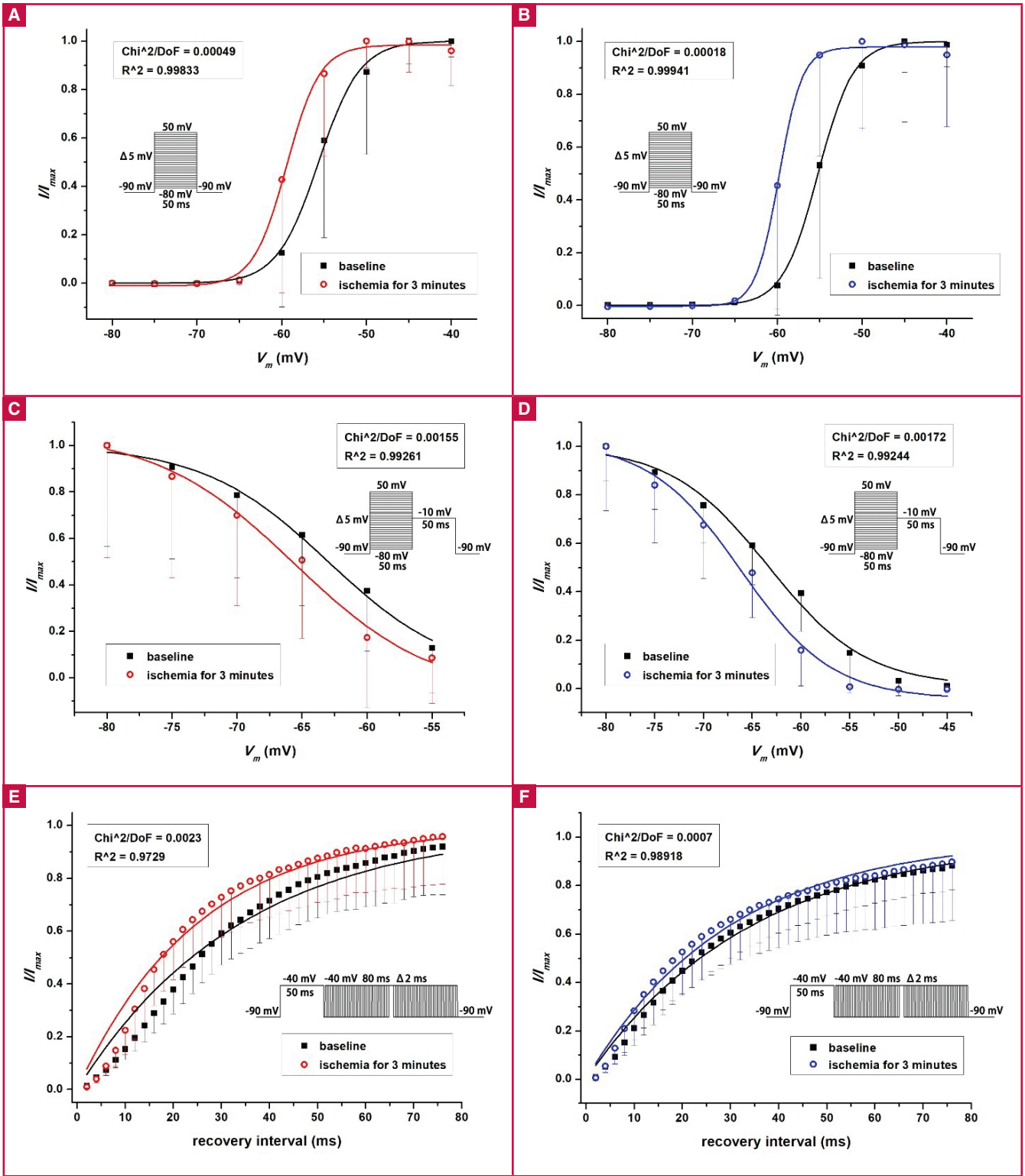

Fig. 5. Gating characteristic curves between baseline and ischaemia for three minutes. (A) Activation curve in the control group. (B) Activation curve in the statin group. (C) Inactivation curve in the control group. (D) Inactivation curve in the statin group. (E) Recovery curve in the control group. (F) Recovery curve in the statin group.

that I_{Na} was gradually attenuated over time after 10 minutes of simulated ischaemia, which was consistent with previous reports.[14] A possible reason may be the secondary damage to cells by secondary calcium overload related to increased intracellular sodium concentration.[15]

The aconitine model has shown[16,17] that the abnormally increased I_{Na} may result in the increase of 0 phase amplitude of the action potential. As the increased action potentials pass into the adjacent tissue in the relative refractory period, it will cause threshold stimulation, which may lead to premature

contraction. In our study, it was observed that I_{Na} had first been increased and then decreased in the simulated ischaemic state. Therefore, in the early stage of ischaemia, cardiomyocytes were in a heterogeneous ischaemic state, and the dispersion of I_{Na} in ischaemic tissue would be increased with the prolongation of ischaemia, which may be one of the bases for the formation of local abnormal current.

As a basic drug of acute coronary syndrome, statins have been shown to reduce the morbidity of ventricular arrhythmias and the mortality rate.[18,19] Therefore we observed the effect of atorvastatin on I_{Na}, which was in the early stage of ischaemia, and found that the increased current was inhibited. As we know, before producing pleiotropic effects, statins should inhibit HMG-CoA reductase and then block the important mevalonate pathway.[20,21] However, Gerber *et al*. showed that atorvastatin decreased the HMG-CoA reductase activity in L cells only after incubation with the drug for 18 hours.[22] In addition, Vaquero *et al*. demonstrated the membrane capacitance was not changed by atorvastatin.[11] Therefore, non-specific perturbation of the membrane seems a very unlikely mechanism for atorvastatin to be responsible for, otherwise the capacitance would be changed as the dielectric constant had been modified.

As a fat-soluble statin, atorvastatin calcium is slightly soluble in pH 7.4 phosphate buffer, which means that the theoretical maximum range of atorvastatin is 82.68 to 826.8 μmol/l. We used a concentration of 5 μmol/l, which was equivalent to the clinical dose of 20–80 mg/d.[23] This could avoid the use of a fat-soluble solvent, which may also influence the membrane currents.

Conclusions

In this study we observed the time-dependent effect of atorvastatin on I_{Na} in a simulated ischaemic condition and found that the phenomenon of transiently increased I_{Na} disappeared. The gated characteristics showed that atorvastatin reduced K_i and weakened the decline of τ value caused by ischaemia. Therefore the channel inactivation was faster and the recovery was slower, which caused the number of open channels per unit time to decrease, finally resulting in a decrease in whole-cell current.

Atorvastatin inhibited the abnormal increase of I_{Na} during the early stage of simulated ischaemia by acting on the processes of inactivation and recovery. As statins can block the activity of a voltage-gated calcium channel,[24] atorvastatin could also transiently block the sodium channel when entering the cell during the first three to seven minutes of ischaemia. Interestingly, atorvastatin appeared to prevent a further decrease in I_{Na} as the ischaemic time extended to more than 19 minutes, indicating another cardioprotective effect of atorvastatin, in preventing further ischaemic injury (such as ischaemic postconditioning of statins[25]). Therefore atorvastatin played a role only as a buffer in abating rapid changes in I_{Na} over time during early ischaemia, which helped to reduce the electrical heterogeneity of the ischaemic myocardium[26,27] and improve the cardiac arrhythmia matrix effect.

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Walnuts may help lower blood pressure for those at risk of heart disease

In a randomised, controlled trial, researchers examined the effects of replacing some of the saturated fats in participants' diets with walnuts. They found that when participants ate whole walnuts daily in combination with lower overall amounts of saturated fat, they had lower central blood pressure.

According to the researchers, central pressure is the pressure that is exerted on organs such as the heart. This measure, like blood pressure measured in the arm in the traditional way, provides information about a person's risk of developing cardiovascular disease (CVD).

Dr Penny Kris-Etherton, distinguished professor of nutrition at Penn State, said the study suggests that because walnuts lowered central pressure, their risk of CVD may have also decreased. 'When participants ate whole walnuts, they saw greater benefits than when they consumed a diet with a similar fatty acid profile as walnuts without eating the nut itself,' Kris-Etherton said. 'So, it seems like there's a little something extra in walnuts that are beneficial – maybe their bioactive compounds, maybe the fibre, maybe something else – that you don't get in the fatty acids alone."

Alyssa Tindall, recent student in Dr Kris-Etherton's lab and a new PhD graduate in nutrition, said the study was one of the first to try to uncover which parts of the walnuts help to support heart health.

'Walnuts contain alpha-linolenic acid (ALA) a plant-based omega-3 that may positively affect blood pressure,' Tindall said. 'We wanted to see if ALA was the major contributor to these heart-healthy benefits, or if it was other bioactive component of walnuts, like polyphenols. We designed the study to test if these components had additive benefits.'

For the study, the researchers recruited 45 participants with overweight or obesity who were between the ages of 30 and 65 years. Before the study began, participants were placed on a 'run-in' diet for two weeks.

'Putting everyone on the same diet for two weeks prior to the start of the study helped put everyone on the same starting plane,' Tindall said. 'The run-in diet included 12% of their calories from saturated fat, which mimics an average US diet. This way, when the participants started on the study diets, we knew for sure that the walnuts or other oils replaced saturated fats.'

After the run-in diet, the participants were randomly assigned to one of three study diets, all of which included less saturated fat than the run-in diet. The diets included one that incorporated whole walnuts, one that included the same amount of ALA and polyunsaturated fatty acids without walnuts, and one that partially substituted oleic acid for the same amount of ALA found in walnuts, without any walnuts.

All three diets substituted walnuts or vegetable oils for 5% of the saturated fat content of the run-in diet. All participants followed each diet for six weeks, with a break between diet periods.

Following each diet period, the researchers assessed the participants for several cardiovascular risk factors, including central systolic and diastolic blood pressure, brachial pressure, cholesterol level and arterial stiffness.

The researchers found that while all treatment diets had a positive effect on cardiovascular outcomes, the diet with whole walnuts provided the greatest benefits, including lower central diastolic blood pressure. In contrast to brachial pressure, which is the pressure moving away from your heart and measured with an arm cuff in the doctor's office, central pressure is the pressure moving toward your heart.

Tindall said that the results underline the importance of replacing saturated fat with healthier alternatives. 'An average American diet has about 12% calories from saturated fat, and all our treatment diets all had about 7%, using walnuts or vegetable oils as a replacement,' Tindall said. 'So, seeing the positive benefits from all three diets sends a message that regardless of whether you replace saturated fats with unsaturated fats from walnuts or vegetable oils, you should see cardiovascular benefits.'

Kris-Etherton added that the study supports including walnuts as part of a heart-healthy diet. 'Instead of reaching for fatty red meat or full-fat dairy products for a snack, consider having some skim milk and walnuts,' Kris-Etherton said. 'I think it boils down to how we can get the most out of the food we're eating, specifically, how to get a little more bang out of your food buck. In that respect, walnuts are a good substitute for saturated fat.'

Source: Medical Brief 2019