Acetylcholine-regulated K^+ current remodelling in the atrium after myocardial infarction and valsartan administration

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BACKGROUND: Atrial fibrillation (AF) is a common complication of myocardial infarction (MI). Angiotensin II receptor antagonists prevent the promotion and propagation of AF. However, the activation of the acetylcholine-regulated K^+ current ($I_{K,ACH}$) in the atrium after MI and the effect of valsartan on $I_{K,ACH}$ are less understood.

METHODS: Twenty-four adult rabbits were randomly divided into three groups: sham-operated, MI and MI plus valsartan administration (MI+valsartan). The sham-operated group received a median sternotomy without left ventricular coronary artery ligation. Both the MI group and the MI+valsartan group received a median sternotomy followed by ligation of the midpoint of the left ventricular coronary artery. The MI+valsartan group was administered oral valsartan for 12 weeks. After 12 weeks, the initiation of AF was measured by vagal stimulation followed by quick excision of the heart. $I_{K,ACH}$ in the left atrial myocardium was measured by the patch clamp technique.

RESULTS: AF was induced in four animals in the MI group, two in the sham-operated and two in the MI+valsartan groups, with the total AF duration expectedly longer in the MI group than in the sham-operated and MI+valsartan groups (38 s versus 9 s and 9 s, respectively). Furthermore, the mean ($\pm$ SEM) density of $I_{K,ACH}$ increased significantly more in the left atrial myocardia of the MI group than in the sham-operated and the MI+valsartan groups (-13 ± 0.42 pA/pF versus -9 ± 0.38 pA/pF and -10 ± 0.37 pA/pF, respectively at -100 mV; and 4.1 ± 0.28 pA/pF versus 3.1 ± 0.27 pA/pF and 3.3 ± 0.27 pA/pF, respectively at 20 mV; $P < 0.05$). However, there was no statistically significant difference in $I_{K,ACH}$ between the sham-operated group and the MI+valsartan group.

CONCLUSIONS: AF is associated with increased $I_{K,ACH}$ after MI. Inhibition of increased $I_{K,ACH}$ may be the mechanism by which valsartan prevents AF following MI.

Key Words: Atrial fibrillation; Myocardial infarction; Rabbit; Valsartan

Le remodelage du courant K^+ induit par l'acétylcholine dans l'oreillette après un infarctus du myocarde et l'administration de valsartan

HISTORIQUE : La fibrillation auriculaire (FA) est une complication courante de l'infarctus du myocarde (IM). Les antagonistes des récepteurs de l'angiotensine II préviennent la promotion et la propagation de la FA. Cependant, on comprend moins l'activation du courant K^+ induit par l'acétylcholine ($I_{K,ACH}$) dans l'oreillette après l'IM et l'effet du valsartan sur $I_{K,ACH}$.

MÉTHODOLOGIE : Vingt-quatre lapins adultes ont été divisés aléatoirement en trois groupes : opération placebo, IM et IM associé à l'administration de valsartan (IM+valsartan). Le groupe ayant subi une opération placebo a reçu une sternotomie médiane sans ligature de l'artère coronaire ventriculaire gauche. Tant le groupe d'IM que le groupe d'IM+valsartan ont subi une sternotomie médiane suivie d'une ligature au milieu de l'artère coronaire ventriculaire gauche. Le groupe d'IM+valsartan a reçu du valsartan par voie orale pendant 12 semaines. Au bout de 12 semaines, l'apparition de FA était mesurée par stimulation vagale suivie d'une excision rapide du cœur. On a mesuré $I_{K,ACH}$ du myocarde auriculaire gauche au moyen de la technique du patch-clamp.

RÉSULTATS : La FA a été induite chez quatre animaux du groupe d'IM, deux de ceux ayant subi une opération placebo et deux du groupe d'IM+valsartan, et comme prévu, la durée totale de la FA était plus longue dans le groupe d'IM que dans le groupe ayant subi une opération placebo ou l'IM+valsartan (38 s par rapport à 9 s et 9 s, respectivement). De plus, la densité moyenne ($\pm$ ET) d' $I_{K,ACH}$ a beaucoup plus augmenté dans le myocarde auriculaire gauche du groupe d'IM que du groupe ayant subi l'opération placebo ou l'IM+valsartan (-13 ± 0.42 pA/pF par rapport à -9 ± 0.38 pA/pF et -10 ± 0.37 pA/pF, respectivement, à -100 mV; et $4,1 \pm 0,28$ pA/pF par rapport à $3,1 \pm 0,27$ pA/pF et $3,3 \pm 0,27$ pA/pF, respectivement, à 20 mV; $P < 0,05$). Cependant, il n'y avait pas de différence statistiquement significative d' $I_{K,ACH}$ entre le groupe ayant subi une opération placebo et le groupe d'IM+valsartan.

CONCLUSIONS : La FA s'associe à une augmentation de $I_{K,ACH}$ après l'IM. L'inhibition de $I_{K,ACH}$ accrue peut être le mécanisme par lequel le valsartan peut prévenir la FA après l'IM.

New-onset atrial fibrillation (AF) is a common complication of myocardial infarction (MI) (1,2). AF in the setting of ST segment elevation MI is estimated to occur in up to 20% of patients, depending on the population studied (3,4). Clinical and experimental evidence suggests that autonomic tone plays an important role in the pathogenesis of AF (5-7). The relationship between the expression of low vagal output and increased sympathetic tone was observed after MI (8). Whether the activation of ion currents, such as the acetylcholine (ACh)-regulated K^+ current ($I_{K,ACH}$), is affected in the atrium after MI is less well understood.

More recent experimental data suggest that angiotensin II receptor antagonists also prevent the promotion and propagation of AF by

suppressing electrical and structural cardiac remodelling (9). Modulation of K^+ currents by angiotensin II has also been reported (10). In the present study, we investigated the changes in $I_{K,ACH}$ and compared the respective current densities in the left atrium of normal and MI rabbits. Furthermore, we observed the effect of valsartan on $I_{K,ACH}$.

METHODS

The protocol of the present study was approved by the Research and Ethics Committee of Wuhan University (Wuhan, Hubei, People's Republic of China). Animal handling was performed according to the Wuhan Directive for Animal Research.

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Preparation of the animal model

Twenty-four adult New Zealand white rabbits (weight 1.5 kg to 2.0 kg) were randomly divided into three groups: the sham-operated group (n=8), the MI group (n=8) and the MI plus valsartan administration (MI+valsartan) group (n=8).

The rabbits were anesthetized in the abdomen with intravenous sodium pentobarbital (30 mg/kg to 40 mg/kg); additional doses were administered when required throughout the experiment. The animals were intubated and ventilated with room air supplemented with low-flow oxygen from a mechanical ventilator. Surgery was performed under sterile conditions. A thoracotomy was performed through the third intercostal space and the heart was exposed after excising the pericardium. The sham-operated group received a median sternotomy without ligation of the left ventricular coronary artery. Both the MI and MI+valsartan groups received a median sternotomy followed by ligation of the midpoint of the left ventricular coronary artery. MI was confirmed by regional cyanosis and electrocardiographic change (more than two ST segment elevations of 0.1 mV or higher). After this, the MI+valsartan group was administered 10 mg/kg/day of oral valsartan (Beijing Nuohua Medicine Co Ltd, China) for 12 weeks.

Experimental protocol

After 12 weeks, the rabbits were anesthetized once more and the double cervical vagosympathetic trunks were severed. An electrocardiogram of leads II and aVF was continuously used to monitor the animals. Silver wires were introduced in the double cranial end of the vagosympathetic trunks toward the heart. Electrical stimulation was delivered at a frequency of 10 Hz, with a 0.2 ms pulse duration and a voltage of 5 V (electrophysiology stimulator SEN-7103; Nihon Kohden, Japan). The voltage chosen for the vagal stimulation (VS) was 5 V higher than the voltage at which a sinus arrest lasting longer than 2 s (complete atrio-ventricular block) was achieved. The initiation of AF was measured. When AF was induced, electrical stimulation was terminated. If the duration of electrical stimulation lasted for 30 s and AF was not induced, electrical stimulation was also terminated.

Patch-clamp techniques

After VS, the hearts were extracted and perfused on a Langendorff apparatus at 37°C by pumping with calcium-free Tyrode's solution. Following a 3 min perfusion with calcium-free Tyrode's solution, the low calcium (100 µmol/L) Tyrode's solution containing 0.40 mg/mL type I collagenase was perfused for approximately 8 min. The heart was then detached from the cannula. The left atria were sliced into small pieces and placed in a beaker containing 1.5 mL of recirculated enzyme solution and 10 mL of calcium-free Tyrode's solution with 1% bovine serum albumin. The left atrial pieces were agitated and incubated in the same solution at 37°C. After 5 min, the cell suspension was filtered through nylon gauze (200 µm mesh) and the calcium concentration was gradually increased to 2 mmol/L within 30 min. Finally, the cells were stored at room temperature for at least 1 h before use.

The isolated cells were perfused with Tyrode's solution containing 136 mM sodium chloride, 5.4 mM potassium chloride, 1 mM calcium chloride, 1 mM magnesium chloride, 10 mM glucose and 10 mM HEPES (pH 7.4). Ionic currents were recorded with whole-cell clamp methods, using an EPC-9 amplifier (HEKA Instruments Inc, USA). The pipette solution contained 110 mM potassium aspartate, 20 mM potassium chloride, 1 mM magnesium chloride, 5 mM magnesium ATP, 0.1 mM GTP, 10 mM EGTA, 5 mM phosphocreatine and 10 mM HEPES (pH adjusted to 7.3 with potassium hydroxide). To record $I_{K,ACh}$, other subtypes of muscarinic ACh receptors were inhibited by using the following subtype-selective antagonists: 100 nM pirenzepine (an M1 blocker), 2 nM 4-diphenylacetoxy-N-methylpiperidine methobromide (an M3 inhibitor) and 200 nM tropicamide (an M4 inhibitor). $I_{K,ACh}$ was induced by 1 µM ACh and recordings of $I_{K,ACh}$ were generally conducted with 1 µM dofetilide and 20 µM chromanol 293B in the bathing solution to block the rapidly and slowly activating

components of the delayed rectifier K^+ current. Contamination from the sodium current was prevented by holding the cell at -50 mV. Cadmium chloride (200 µM) was used to inhibit the Ca^{2+} current as well as the Ca^{2+} -activated chloride current. ATP-sensitive K^+ current, if any, was suppressed by 10 µM glyburide in the perfusate and 5 mM magnesium ATP in the pipette (11). $I_{K,ACh}$ was induced by 1 µM ACh in the bathing solution and defined as the 1 µM atropine-sensitive current to exclude contamination from the background inward rectifier K^+ current.

Statistical analysis

Data were analyzed using SPSS 11.0 (SPSS Inc, USA). Values are expressed as mean ± SEM. Statistical comparisons were made using ANOVA. Paired and unpaired comparisons were conducted using Student's *t* test. Statistical significance was assumed for $P < 0.05$.

RESULTS

Initiation of AF

In the sham-operated group, AF was induced in two rabbits. AF duration was 3 s in one rabbit and 6 s in the other. In the MI group, AF was induced in four rabbits. AF duration was 7 s, 9 s, 10 s and 12 s in the four rabbits, respectively. In the MI+valsartan group, AF was induced in two rabbits. AF duration was 4 s in one rabbit and 5 s in the other.

Correlation of $I_{K,ACh}$ density in the three groups

$I_{K,ACh}$ was induced by 1 µM ACh in the bathing solution and defined as the 1 µM atropine-sensitive current to exclude contamination from the background inward rectifier K^+ current. $I_{K,ACh}$ demonstrated strong inward rectification with increasing inward currents during hyperpolarizing voltage steps and rapidly decaying outward currents during depolarization. The amplitude of $I_{K,ACh}$ was measured at the end of the 2 s voltage steps. $I_{K,ACh}$ determined at 2 s had stronger inward rectification with smaller outward currents on membrane depolarization. As illustrated, the density of $I_{K,ACh}$ was substantially lower in the left atrial myocytes from the sham-operated and MI+valsartan groups than in those from the MI group (-9 ± 0.38 pA/pF and -10 ± 0.37 pA/pF versus -13 ± 0.42 pA/pF, respectively at -100 mV; and 3.1 ± 0.27 pA/pF and 3.3 ± 0.27 pA/pF versus 4.1 ± 0.28 pA/pF, respectively at 20 mV; $P < 0.05$). In the sham-operated and MI+valsartan groups, $I_{K,ACh}$ showed an increasing trend, but this did not reach statistical significance (Figure 1).

DISCUSSION

AF can occur secondary to postinfarction complications. However, when it occurs independently, it carries a worse prognosis (2,12). There is considerable interest in the role of the renin-angiotensin-aldosterone system in the development of AF (13). Recent reports have suggested that there is a reduction in the development or recurrence of AF in patients treated with angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists (14,15). This may be a novel approach to the management of AF.

Kumagai et al (16) showed that sustained AF induction in dogs with uncontrolled ventricular rates caused left ventricular dysfunction. Candesartan started one week before the onset of tachypacing, and continued throughout tachypacing, reduced AF duration. Atrial effective refractory period shortening was unaffected, indicating benefit independent of rate-dependent remodelling. In another study, Li et al (17) demonstrated that candesartan reduced interstitial fibrosis, suggesting that efficacy against AF was due to prevention of tachycardiomyopathy-induced structural remodelling. Studies suggest that the renin-angiotensin-aldosterone system could affect ion channels, action potential period and impulse propagation, and facilitate re-entry. Intracellular angiotensin II reduced the L-type Ca^{2+} current in rat ventricular cells, whereas the opposite was observed in hamsters (18). Angiotensin II increases the L-type Ca^{2+} current through protein kinase C-dependent pathways (19). The T-type Ca^{2+} current is increased by angiotensin II stimulation (20). The rapidly activating component of

the delayed K^+ rectifier current in guinea pig ventricular myocytes is increased by clinically relevant concentrations of angiotensin II (30 nmol/L), whereas the slow component of the current is decreased (10).

It is clear that MI can alter ion channel function. In rabbits at eight weeks post-MI, it appears that the abnormally prolonged action potential duration is accompanied not only by a reduction in L-type Ca^{2+} current activity but also an increase in Na^+/Ca^{2+} exchanger current density (21). Compared with myocytes from sham-operated rats, post-MI myocytes showed significant reductions in transient outward K^+ current density, inward rectifier K^+ current density and resting membrane potential (22). However, no studies have reported the changes that occur in $I_{K,ACh}$ in the atrium after MI. In the present study, we found that $I_{K,ACh}$ increased after MI. However, after 12 weeks of valsartan administration, there was no change in $I_{K,ACh}$ between the sham-operated and MI+valsartan groups. These preliminary results may show that oral valsartan can inhibit increases in $I_{K,ACh}$ after MI. Other studies have shown that there is an imbalance between sympathetic and vagal output to the heart after MI (23). Increased sympathetic activity is associated with a high risk of malignant ventricular arrhythmias during myocardial ischemia (24). We suppose that after MI, there is an increase in sympathetic tone with parasympathetic withdrawal. The increase in $I_{K,ACh}$ may be a response to decreased vagal tone. The mechanism for the effect of valsartan on $I_{K,ACh}$ is not clear. Our results have qualitatively shown that increased $I_{K,ACh}$, rather than decreased vagal input, may be the important factor responsible for AF induction after MI. Furthermore, in our study, oral valsartan inhibited increases in $I_{K,ACh}$ after MI and significantly reduced AF induction due to VS. The present study therefore serves as a preliminary investigation of this important field, and should be followed by larger clinical trials.

Limitations

The present study has several limitations. First, due to the difference in the number of animals in which AF was induced in the three groups, we did not perform a statistical analysis of the difference in AF duration observed between the groups. Our findings therefore lacked the power to conclude on any statistical significance.

Second, we ligated the medial left anterior descending artery, a branch of the left coronary artery, to make an animal model of anterior MI. Studies have shown that AF occurring 24 h or more after the onset of acute MI tend to be associated more with anterior MI (25). Occlusion of the medial left anterior descending artery would be expected to cause heart failure to some extent. Sinno et al (26) demonstrated increased AF susceptibility due to slowing of local conduction in ischemic atrial remodelling. It is well known from human studies that most patients who develop AF after myocardial infarction have lost a significant amount of cardiac muscle and AF is a sequel of heart failure. However, there were no significant differences in the signs of lethargy, loss of appetite, dyspnea and peripheral edema between the MI group and sham-operated group. We failed to measure hemodynamic parameters and B-type natriuretic peptide levels.

Third, Dobrev et al (27) showed that chronic human AF induces down-regulation of $I_{K,ACh}$ and attenuates the muscarinic receptor-mediated shortening of action potential duration. Earlier studies found that atrial tachycardia remodelling increases the tertiapin-Q-sensitive atrial current. In the present study, we did not investigate the changes in the tertiapin-Q-sensitive atrial current and the constitutively active $I_{K,ACh}$ after MI in rabbits. It would be interesting to investigate changes in constitutively active $I_{K,ACh}$ after MI.

Finally, other studies showed that treatment with valsartan, or the combination of captopril plus valsartan, resulted in similar changes in cardiac volume and infarct segment length after MI (28). However, in the present study, we did not investigate the effect of valsartan on MI defects in rabbits with induced AF and those without AF. Whether the effect of valsartan on MI defect is related to the induced AF requires further investigation.

Figure 1 **A** Comparison of acetylcholine (ACh)-regulated K^+ current ($I_{K,ACh}$) in the sham-operated group, the myocardial infarction (MI) group and the MI plus valsartan administration (MI+valsartan) group. Two-second voltage steps were delivered to elicit $I_{K,ACh}$ with potentials ranging from -100 mV to $+50$ mV. The same voltage protocols were applied throughout the study. $I_{K,ACh}$ was activated in the presence of $1 \mu M$ ACh. The dashed line indicates the zero current level. **B** The density-voltage relationship of the currents (seven cells for the sham-operated group, seven cells for the MI group and eight cells for the MI+valsartan group). The density (vertical axis) was measured in pA/pF. The voltage (horizontal axis) was measured in mV. $I_{K,ACh}$ was measured at the end of the 2 s pulses. The density of $I_{K,ACh}$ in the MI group was higher than in the sham-operated and MI+valsartan groups

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