Laboratory Research

Effect of cyclosporine-A on electrophysiological properties of atria in tachycardia-induced atrial fibrillation

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Objective  To investigate the effects of cyclosporine-A (CsA), a calcineurin (CaN) inhibitor, on electrophysiological properties of atria in canine tachycardia-induced model of AF. Methods  Eighteen healthy adult mongrel canines weighing 17.0 to 23.2 kg (ranged from 2 to 4 years old) were randomized to 3 groups, Sham group (no pacemaker was implanted), atrial tachypacing group (ATP group) and CsA group (atrial tachypacing plus oral administration of CsA 10 mg · kg⁻¹ · d⁻¹). Electrophysiological tests were performed on each group at baseline and after 8 weeks' tachypacing. Measurements included atrial effective refractory period (AERP), conduction velocity (CV), wave length (WL), atrial fibrillation load and rate-adaptability. Results  After 8 weeks' atrial tachypacing, ATP and CsA groups showed significant longer duration of the P wave, shorter AERP, decreased adaptation of AERP, slower CV, shorter WL and longer AF duration compared to the sham group (all P<0.05). AERP of the CsA group was longer than that of ATP group (P<0.05), but there were no differences in rate-adaptability, CV, incidence of induced AF and AF duration between CsA group and ATP group. Conclusions  Our results suggest that calcineurin pathway intervention by CsA have a positive effect on tachycardia-induced electrical remodeling of atria, but can not prevent or reverse AF. (J Geriatr Cardiol 2008; 5:175-178)

Key words  atrial fibrillation; calcineurin; CsA; electrophysiology; electrical remodeling

Introduction

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice. Our understanding of the pathophysiology of atrial fibrillation has increased tremendously over the past few years. The importance of electrical remodeling and structural remodeling has been widely appreciated and has opened new avenues for pharmacological research. Intracellular calcium-activated calcineurin is a protein phosphatase that dephosphorylates nuclear transcription factors, which translocate to the nucleus and promote increased expression of hypertrophic marker genes. In recent years, several studies found calcineurin (CaN) signaling pathway was involved in the activation and maintenance of AF. We therefore hypothesized that inhibition of activation of CaN signaling pathway might prevent or reverse AF. In this study we investigated the effect of cyclosporine-A (CsA), a blocker of calcineurin, on electrophysiological properties of atria in tachycardia-induced AF model in canines, including atrial effective refractory period (AERP), conduction velocity (CV), wave length (WL), atrial fibrillation load and rate-adaptability, et al.

Animal preparation

Eighteen healthy mongrel canines (17.0–23.2 kg, aged from 2.0 to 4.0 years, provided by the Animal Center of Chinese General PLA Hospital) were anesthetized with pentobarbital (15mg · kg⁻¹ · iv). Animal-handling procedures followed guidelines of the National Institutes of Health of the USA. Unipolar pacing leads were inserted through jugular veins into right atrial appendage under fluoroscopic guidance, and connected to VOO pacemakers (Fudan University, China) in subcutaneous pockets in the neck. A bipolar electrode was inserted into the RA for stimulating and recording during serial closed-chest electrophysiological studies (EPSs). After 24-h post-operative recovery, a baseline closed-chest EPS was performed and then 8-week ATP at 400 bpm was instituted. Closed-chest EPS was repeated after 8 weeks of ATP under intravenous pentobarbital anesthesia.

Study protocol

Study animals were randomly divided into 3 groups. Sham group, with no pacemaker implanted and no other interventions (n=6). Atrial tachypacing group (ATP group) had high speed pacemaker implanted after anesthesia with pentobarbital iv. After 24h post-operative recovery, the pacemaker was started to work at a rate of 400 bpm for 8 weeks (n=6). CsA group(n=6) was treated as ATP group except...
CsA (10 mg · kg⁻¹ · d⁻¹) was administrated orally from ATP onset to the morning of the final closed-chest EPS.

Electrophysiological study

For closed-chest EPS, dogs were anesthetized with pentobarbital (10–20 mg/kg) and ventilated mechanically. The atrial tachypacemaker was deactivated and effective refractory period (ERP) of the RA appendage was measured at basic cycle lengths (BCLs) of 150, 200, 250, 300, and 360 ms with 10 basic stimuli (S1) followed by premature extrastimuli (S2) with 5-ms decrements. All stimuli were twice threshold, 2-ms pulses. The longest S1–S2 interval failing to capture defined the ERP. AF was induced with 1–10 s burst pacing (10 Hz, 4×threshold current). To estimate mean AF duration in each dog, AF was induced 10 times for AF duration <20 min and 5 times for 20–30 min AF. Prolonged AF (>45 min) was terminated by direct current (DC) electrical cardioversion. A 30-min rest period was then allowed before continuing measurements. If prolonged AF was induced twice, no further AF induction was performed.

Right atrial activation conduction velocity (CV) was calculated. Four electrodes were placed on high right atrium, two of which were used to pace the right atrium with 300 ms duration. The right atrium CV was calculated by distance between the two pairs of electrodes of another mapping catheter divided by time of atrial conduction. Wave length was calculated by atrial effective refractory period (AERP) × CV with BCL of 300 ms. Rate-adaptation was determined by subtracting the ERP at a BCL of 150 ms from the value at a BCL of 300 ms in each dog. The greater value means better adaptability.

AF was induced by initial procedures at BCLs of 150 and 300 ms with premature stimuli S2 and S3 to S4 with 5 ms decrements. The duration of S1 and S2 was 280ms and 140ms respectively. S3 was equal to AERP+30 ms. If AF was not induced by previous premature stimuli, S4 was used according to procedure of S3. Chronic AF was defined with irregular atrial repeated response with the duration >15 min. AF was induced with 10 s burst pacing (10Hz, 4×threshold current, 2 ms pulses) and recorded incidence of induced AF by 10 stimuli and duration. If AF sustained over 45 minutes, direct current electrical cardioversion was given. Stimuli restarted after 30 mins recovery. If continuous AF (>45 min) presented, then stimulation was stopped. AF induced by stimuli caused by catheter was considered invalid and not recorded.

Statistical analysis

Data are presented as means±SD. Comparison of continuous variables was performed with unpaired Student’s t test and categorical variables were compared by Pearson’s Chi-Square test. Statistical significance was accepted when P was <0.05. Statistical analysis was performed using SPSS (version 13.0) software.

Results

All the experiments were successfully completed except for one dog in the sham group, which died from ventricular fibrillation.

P-wave duration on the surface ECG

There were no differences of mean P wave duration between the 3 groups at baseline. However, at 8 wks, the mean P wave duration of both ATP and CsA groups was significantly prolonged compared with that of sham group (P<0.05), which suggested atrial tachypacing lead to electrical remodeling and decrease in transmission velocity. No significant difference between ATP and CsA groups was found (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Sham(n=6)</th>
<th>ATP(n=6)</th>
<th>CsA(n=6)</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>51.57 ± 6.06</td>
<td>49.33 ± 5.70</td>
<td>51.87 ± 6.77</td>
</tr>
<tr>
<td>8 Weeks</td>
<td>50.58 ± 3.97</td>
<td>74.36 ± 10.11</td>
<td>71.98 ± 8.76</td>
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</table>

*Compared with Sham group, P<0.05; * compared with baseline, P<0.05

Atrial effective refractory period (AERP)

After 8 weeks’ of tachypacing AERP of the ATP and CsA significantly decreased compared with that of sham and baseline (P<0.05), especially at BCL 300ms, which suggested the rate-adaptation of the atria decrease. AF was more easily induced by AERP at lower rate. Compared with that of ATP group, AERP of CsA group was decreased at BCL of 300ms (P<0.05) but remained similar at BCL of 150 ms (P>0.05) (Table 2).

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<thead>
<tr>
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<th>Sham(n=6)</th>
<th>ATP(n=6)</th>
<th>CsA(n=6)</th>
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<tbody>
<tr>
<td>BCL 300</td>
<td>120.33 ± 5.99</td>
<td>100.17 ± 7.88</td>
<td>119.83 ± 4.07</td>
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<tr>
<td>BCL 150</td>
<td>103.17 ± 4.31</td>
<td>122.67 ± 5.35</td>
<td>102.32 ± 5.40</td>
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<tr>
<td>BCL 150</td>
<td>88.33 ± 5.75</td>
<td>74.67 ± 6.68</td>
<td>97.33 ± 9.91</td>
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</table>

*Compared with sham, P<0.05; * compared with baseline, P<0.05

Atrial conduction velocity (CV)

Compared with those in the sham group, atrial CV in
the ATP and CsA group was decreased ($P<0.05$) with no difference between latter two groups, which suggested CsA have no effect on tachycardia-induced electrical remodeling of atrial.

**Atrial wave length (WL)**

The results of atrial WL were shown on Tables 3 and 4. Compared with those in the sham group, WL of ATP and CsA groups were significantly decreased ($P<0.05$), but there was no difference between these two groups. Inhibition of CaN signal pathway caused by CsA had little effect on atrial WL.

**Discussion**

In this study, we investigated the effects of Cyclosporine-A (CsA), a calcineurin inhibitor, on electrophysiological properties of atria in canine tachycardia-induced model of AF. We found that calcineurin pathway intervention by CsA has a positive effect on tachycardia-induced electrical remodeling of atria, but can not prevent or reverse AF.

Calcineurin enzyme activity is activated and expression of the downstream signal nuclear factor of activated thymocytes (NFAT) is augmented in pigs subjected to 6 weeks of atrial tachypacing. Calcineurin mRNA expression is also increased in atria of AF patients. Based on this information, we speculated that the calcineurin inhibitor cyclosporine-A would inhibit atrial tachycardia remodeling. As a result, we were unable to demonstrate any protective effect of cyclosporine-A against AF onset or maintenance. Our results are consistent with previous animal observations of CsA efficacy in AF prevention. However, we also found that calcineurin pathway intervention by CsA has a positive effect on tachycardia-induced electrical remodeling of atria. AERP of the CsA group was longer than that of ATP group, but there were no differences in rate-adaptability, CV, incidence of induced AF and AF duration between CsA group and ATP group.

Studies showed that decrease in AERP in AF was associated with down-regulation of density of $I_{CaL}$ and the mRNA expression of these receptors and of the L-type Ca$^{2+}$-channel subunits. Brundel et al. found AF is predominantly accompanied by decreased protein contents of the L-type Ca$^{2+}$ channel and several potassium channels. Reductions in L-type Ca$^{2+}$ channel correlated with AERP and rate adaptation, and they represent a probable explanation for the electrophysiological changes during AF. Our other studies also showed that CsA could increase the mRNA expression of $\alpha_{1C}$ subunit of L-type Ca$^{2+}$-channel, which might be the pathway of effect of CsA on electrical remodeling caused by quick atrial pacing.

This study is to our knowledge the first to assess the effects of calcineurin inhibitors (CsA) in an animal model of chronic AF promotion by atrial tachycardia. Our results show that CsA could inhibit the decrease in AERP caused by tachypacing, but no effect on rate-adaptation, AF duration, wavelength, conduction velocity and mean P-wave. This results suggest that AF be a complex pathophysiological process and there be many mechanisms involved in the maintenance and occurrence, which means AF could not be prevented by intervention of single pathway. On the other hand, there might be other pathway also involved in AF, which is still unclear.

The dosage of CsA was selected based on previous studies of CsA use in a dog model. However, we did not

### Table 3 Comparison of atrial CV (mean±SD, cm/s)

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<tr>
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<th>CsA (n=6)</th>
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<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
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<tr>
<td>108.17 ± 7.08</td>
<td>106.67 ± 7.84</td>
<td>111.33 ± 3.33</td>
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<tr>
<td>8 Weeks later</td>
<td></td>
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<tr>
<td>109.67 ± 5.65</td>
<td>79.83 ± 5.42*</td>
<td>82.23 ± 3.74*</td>
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*Compared with sham, $P<0.05$; * compared with baseline, $P<0.05$

### Table 4 Comparison of atrial WL (mean±SD, cm)

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<th>CsA (n=6)</th>
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<tbody>
<tr>
<td>Baseline</td>
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<tr>
<td>12.97 ± 1.09</td>
<td>12.84 ± 1.16</td>
<td>13.66 ± 0.81</td>
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<tr>
<td>8 Weeks later</td>
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<tr>
<td>13.17 ± 0.99</td>
<td>7.04 ± 0.44*</td>
<td>7.99 ± 0.91*</td>
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*Compared with sham, $P<0.05$; * compared with baseline, $P<0.05$

### Table 5 Comparison of atrial rate-adaptation (mean±SD, ms)

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<th>CsA (n=6)</th>
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<tr>
<td>Baseline</td>
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<td></td>
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</tr>
<tr>
<td>20.17 ± 2.71</td>
<td>16.67 ± 3.56</td>
<td>20.67 ± 1.63</td>
<td></td>
</tr>
<tr>
<td>8 Weeks later</td>
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<tr>
<td>20.50 ± 5.01</td>
<td>0.67 ± 1.87*</td>
<td>4.17 ± 2.64*</td>
<td></td>
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</table>

*Compared with sham, $P<0.05$; * compared with baseline, $P<0.05$
monitor the mean CsA concentration in our dogs, as a result, we could not conclude that if higher dosage of CsA had positive effect on prevention or reverse of AF.

Conclusions

Our results suggest that calcineurin pathway intervention by CsA have a positive effect on tachycardia-induced electrical remodeling of atria, but not prevent or reverse AF. We need to discover new insights into the mechanisms and pharmacological prevention of AF.

Acknowledgments

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References