Inflammation abnormalities and inducibility of atrial fibrillation after epicardial ganglionated plexi ablation

Inflammation et induction d’une fibrillation atriale après ablation épicardique de plexus ganglionnaire

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Summary
Background. — Epicardial ganglionated plexi (GP) ablation can prevent atrial fibrillation inducibility. However, the long-term effects of GP ablation on atrial fibrillation have not been elucidated.
Methods. — Thirteen adult dogs of either sex, weighing 13–17 kg, were randomly assigned to a sham-operated group (\(n=6\)) or a GP ablation group (\(n=7\)). After right thoracotomy, the atrial effective refractory period (AERP) was measured and atrial fibrillation was induced by right atrial rapid burst pacing. Atrial fibrillation and AERP were remeasured after anterior right and inferior right GP ablation in the GP ablation group. The animals were allowed to recover for 8 weeks, after which atrial fibrillation and AERP were measured again. Concentrations of C-reactive protein, tumour necrosis factor-alpha (TNF-\(\alpha\)) and interleukin-6 were measured in the blood and atrial tissues.

Abbreviations: AERP, atrial effective refractory period; AF, atrial fibrillation; CRP, C-reactive protein; dAERP, dispersion of atrial effective refractory period; GP, ganglionated plexi; IL, interleukin; TNF-\(\alpha\), tumour necrosis factor-alpha.

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Background

The autonomic nervous system plays an important role in the pathogenesis of AF, with increased sympathetic and parasympathetic activity and a decreased AERP [1–4]. Recent reports have demonstrated that stimulation of the epicardial fat pads containing clusters of GP can initiate spontaneous AF [5]. Radiofrequency GP ablation can prevent AF inducibility [6]. Although long-term vagal denervation of the atria can be achieved by radiofrequency catheter ablation of epicardial fat pads [7], the long-term effects of GP ablation on AF are yet to be clarified. One study [8] reported that radiofrequency fat pad ablation may not achieve long-term suppression of AF induction in a canine model. In humans, a high AF recurrence rate was observed during follow-up after selective vagal denervation alone [9], and GP ablation shows inferior clinical outcome compared with circumferential ablation [10].

Previous studies have demonstrated that inflammation might be an independent risk factor for AF and the nervous system reflexively regulates the inflammatory response, just as it controls heart rate and other vital functions [11]. Recently, a study showed that selective vagal denervation of the sinus and atria decreased heart rate variability and eliminated baroreflex sensitivity [12]. We hypothesized that long-term atrial denervation might induce changes in inflammatory factors, which could be the mechanism of recurrence of AF.

Methods

Preparation of animal model

All animal studies were reviewed and approved by the ethical committee of Wuhan University. Animal handling was carried out according to the Wuhan Directive for Animal Research. Thirteen adult dogs of either sex, weighing 13–17 kg, were used in the present study. The animals were abdominally anaesthetized with pentobarbital sodium (30 mg/kg) and ventilated with room air. Continuous electrocardiogram monitoring was done using lead II and aVF. Dogs in the sham-operated group (group 1; n = 6) underwent right thoracotomy at the fourth intercostal space without fat pad ablation, while dogs in the GP ablation group (group 2; n = 7) underwent fat pad ablation after the right thoracotomy.

Results. — After 8 weeks, atrial fibrillation was induced in all animals in the GP ablation group. AERP and dispersion of AERP (daERP; maximum AERP minus minimum AERP) were increased after GP ablation but AERP recovered after 8 weeks. There were no significant differences in the concentrations of C-reactive protein, TNF-α or interleukin-6 in venous blood between the two groups and the concentration of C-reactive protein in the atrium did not change before and after GP ablation. However, the concentrations of TNF-α and interleukin-6 in the atrium increased significantly 8 weeks after GP ablation (P < 0.05).

Conclusion. — Increased concentrations of TNF-α and interleukin-6 in the atrium after GP ablation provide a new causative factor in terms of atrial fibrillation vulnerability.
After surgery, the fat pad containing the anterior right GP and the inferior right GP were exposed in the GP ablation group [13]. Complete ablation of each GP was achieved as previously described [14].

**Electrophysiological measurements**

AERP was determined by a LEAD-2000B instrument (Sichuan, P. R. China). The $S_1$—$S_2$ intervals were decreased from 150 ms to refractoriness, initially by decrements of 10 ms ($S_1$: $S_2$ = 8:1). As the $S_1$—$S_2$ intervals approached the AERP, decrements were reduced to 5 ms. An extra stimulus ($S_2$) was added in atrial late diastole and the interval between $S_1$ and $S_2$ was reduced in 5 ms steps until there was no propagated atrial response. The longest $S_1$—$S_2$ coupling interval that failed to result in a propagated atrial response was taken as the local AERP. AERP was determined at each electrode pair along the catheters positioned at the right atrial appendage, right atrium, left atrial appendage and left atrium.

An $S_1$-$S_1$ (120 ms cycle length, 5 s) programmed stimulus method was adopted to induce AF. AF was defined as irregular atrial rates faster than 500 beats per minute associated with irregular atrioventricular conduction lasting >5 s. Induction of AF was assessed three times in each dog. In the sham-operated group, AERP and AF were measured immediately after thoracotomy and after 8 weeks. In the GP ablation group, AERP and AF were measured before GP ablation, immediately after GP ablation and 8 weeks after GP ablation.

**Radioimmunoassay**

For radioimmunoassay, 4 mL of venous blood were collected in ethylene diamine tetra-acetic acid vacutainers and centrifuged at 3000 rpm for 10 min at 4 degrees (Avanti J-E, Beckman Coulter, Inc., Brea, CA, USA), before surgery and after 8 weeks. The serum was separated and kept in microtubes and stored at −80°C until assay. After 8 weeks, the hearts were immediately excised and 100 mg of myocardial tissue were taken from the posterior wall of the left and right atria, about 1 cm from the ablation site. Tissue samples were homogenized in phosphate-buffered saline buffer and centrifuged at 3000 g for 20 min at 4°C. After centrifugation, supernatants were collected. The proinflammatory markers CRP, TNF-α and IL-6 were measured using commercially available enzyme linked immunosorbent assay kits (eBioscience, San Diego, CA, USA).

**Statistical analysis**

Values are shown as means ± standard deviations. Statistical comparisons were made using analysis of variance. Paired and unpaired comparisons were made using Student’s t test. Unpaired t tests were used to compare the means of nerve densities. Statistical significance was assumed if p values were less than 0.05.

**Results**

**AERP and induction of AF**

Immediately after anterior right and inferior right GP ablation in the GP ablation group, AERP and dispersion of AERP (dAERP) were significantly longer than before GP ablation. For instance, the AERP at right atrial sites increased from 132 ± 4 ms at baseline to 139 ± 6 ms ($P < 0.05$). After 8 weeks, however, AERP was shorter than immediately after GP ablation, with no statistical significance compared with baseline. There were also no significant differences in AERP and dAERP between
baseline and after 8 weeks in the sham-operated group (Fig. 1A–C).

In the sham-operated group, AF was not induced immediately after thoracotomy or after 8 weeks. Furthermore, rapid burst pacing did not induce AF before GP ablation and immediately after GP ablation, in the GP ablation group. After 8 weeks, AF was induced in all the animals in the GP ablation group. Three episodes of AF were observed in two dogs and two episodes of AF were observed in four dogs. The duration of AF was 38 s, 25 s, 22 s, 19 s, 17 s, 15 s and 12 s in the seven dogs, respectively. Fig. 2 A and B and Table 1 show typical examples of AF induced by rapid pacing and induced AF in the two groups.

Changes in inflammatory markers

In both the sham-operated and GP ablation groups, the blood concentrations of CRP, TNF-α and IL-6 were not significantly different before thoracotomy and after 8 weeks. After 8 weeks, there were no significant differences in CRP concentrations in left and right atrial tissues between the two groups. However, the concentrations of TNF-α and IL-6 in left and right atrial tissues were greatly raised in the GP ablation group compared with the sham-operated group after 8 weeks (TNF-α and IL-6 concentrations in left atrial tissue: 1.4 ± 0.5 vs 3.3 ± 0.6 ng/mg and 0.8 ± 0.4 vs 1.5 ± 0.6 ng/mg, respectively; TNF-α and IL-6 concentrations in right atrial tissue: 1.3 ± 0.5 vs 4.7 ± 0.7 ng/mg and 0.9 ± 0.3 vs 1.8 ± 0.5 ng/mg, respectively; all p values < 0.05) (Fig. 3A and B).

Discussion

The results of the present study show that AF was easily induced by atrial rapid pacing 8 weeks after anterior right and inferior right GP ablation, and that partial atrial denervation can induce an increase in the concentrations of TNF-α and IL-6 in the atrial tissues. These results suggest that there was an increased inflammatory response in the atrium after atrial GP ablation. Although the autonomic effect was eliminated after atrial GP ablation, the increase in inflammatory factors in the atrium may provide a new causative factor in terms of AF vulnerability.

Recently, AF ablation targeting the intrinsic cardiac autonomic nervous system has been shown to improve the success rates of AF ablation [15,16]. Nademanee et al. [17] observed that ablation of atrial sites where rapid, irregular and fractionated electrogram activation occurs, decreases the risk of AF recurrence. The rapid activation may be as a result of local vagal influence [18]. Ablation of these sites may be successful because of the associated changes in autonomic substrate. However, the innervation in the atrium is physiological; whether atrial denervation could induce atrial structural and functional disturbance is yet to be investigated.

Studies have suggested that inflammation leads to ‘atrial myocarditis’ with subsequent electrical and structural atrial changes, resulting in initiation and maintenance of AF [19]. Sata et al. [20] provided insight into the role of the inflammatory process in AF and suggested that inflammation might be an independent risk factor for AF. Several studies have investigated the relationship between IL-6, TNF-α and AF; they found increased concentrations of IL-6 and TNF-α in patients with AF compared with in healthy controls [21,22]. The present study showed that the concentrations of IL-6 and TNF-α from the left and right atria increased 8 weeks after GP ablation. Increased concentrations of IL-6 and TNF-α in the atrium may provide a substrate for AF vulnerability. Recent insights have identified a basic neural pathway that adjusts the inflammatory response. Stimulation of the vagus nerve significantly downregulates the production of IL-6 and TNF-α. We found that the concentrations of IL-6 and TNF-α increased in the left and right atria after GP ablation. Atrial denervation attenuates the inhibiting effect of cholinergic neurons on the anti-inflammatory pathway, hence inducing an increase in IL-6 and TNF-α concentrations.

Recent evidence has demonstrated that an elevated CRP concentration may predict AF in patients [19]; CRP concentrations were significantly increased to a median of 49 days after AF ablation. However, in the present study, we found that the CRP concentration in venous blood and atrial tissues
Table 1  Characteristics of atrial fibrillation induction.

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<th>Sham-operated group</th>
<th>GP ablation group</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>After 8 weeks</td>
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<tr>
<td>Number of animals</td>
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<tr>
<td>Number with sustained AF (&gt; 5 s)</td>
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<td>0</td>
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<td>Mean duration of AF (s)</td>
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<td>8 weeks after ablation</td>
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<td></td>
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<td>7</td>
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<tr>
<td>Mean duration of AF (s)</td>
<td>0 ± 8.6*</td>
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AF: atrial fibrillation. *P < 0.001 vs baseline.

Figure 3.  A. Concentrations of tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP), respectively, in venous blood; there were no significant differences in the concentrations of TNF-α, IL-6 and CRP between the two groups. B. Concentrations of TNF-α, IL-6 and CRP, respectively, in atrial tissues; concentrations of TNF-α and IL-6 in left and right atrial tissues increased 8 weeks after ganglionated plexi (GP) ablation, while concentrations of CRP did not change after 8 weeks. ▲ P < 0.05 vs sham-operated group. LA; left atrium; RA; right atrium.
did not change after GP ablation. A similar elevation in CRP concentration was not seen in patients undergoing supraventricular tachycardia ablation [23]. These studies showed that the rise in CRP concentration was not due to the ablation per se, but rather was something unique to the extensive atrial ablation. The present study showed that the concentrations of IL-6 and TNF-α increased in the atrium. These data suggest that the increases in IL-6 and TNF-α concentrations are not related to atrial ablation but rather to atrial denervation.

In our previous study, we found that the concentration of atrial natriuretic peptide increased in the atrial tissues 8 weeks after GP ablation but the data did not attain statistical significance in the left atrium. Atrial natriuretic peptide has been shown to shorten atrial conduction time and the AERP, which provides a potential electrophysiologic substrate for arrhythmia [24,25]. In the present study, although AERP and dAERP increased after GP ablation, AERP had recovered 8 weeks after GP ablation. We suspect that the changes in AERP and dAERP may be due to the effects of increased atrial natriuretic peptide in the atrium.

We also found that rapid burst pacing did not induce AF before and immediately after GP ablation in the GP ablation group but was induced in all the animals after 8 weeks without vagal stimulation. Increased dAERP may play an important role in induced AF. However, Hirose et al. found that right pulmonary vein—atrial junction ablation increased the incidence of AF during vagal stimulation; they concluded that partial right atrial vagal denervation facilitated initiation of vagally mediated AF. In the present study, the incidence of AF was not related to partial right atrial denervation but to increased concentrations of IL-6 and TNF-α in the atrium.

Study limitations

The present study has several limitations. First, we did not observe the changes in inflammatory factors immediately after GP ablation or after the right thoracotomy procedure. There may have been an increase in CRP concentration after the right thoracotomy procedure. We observed that there was a trend towards an increase in CRP concentrations after 8 weeks, but the data did not attain statistical significance in the two groups. Second, after the procedure, we did not perform any heart rate variability analysis to determine that vagal modification was actually reached. Previous studies have demonstrated that selective vagal denervation by fat pad ablation decreased heart rate variability, and that long-term vagal denervation of the atria can be produced by radiofrequency catheter ablation of epicardial fat pads. Third, a comparison of cytokine concentrations between an atrial ablation group and a GP ablation group should be carried out.

Conclusion

Atrial GP ablation can induce increased concentrations of IL-6 and TNF-α in the atrium. The changes in IL-6 and TNF-α in the atrium provide a new causative factor in terms of AF vulnerability.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References