Botulinum Toxin Injection in Epicardial Autonomic Ganglia Temporarily Suppresses Vagally Mediated Atrial Fibrillation

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Background—Autonomic denervation may suppress atrial fibrillation (AF) vulnerability. This study was designed to assess the short- to mid-term effects of botulinum toxin, a cholinergic neurotransmission blocker, on AF inducibility.

Methods and Results—A total of 23 mongrel dogs were studied. The sinus node and atrioventricular node epicardial fat pads were exposed through a right lateral thoracotomy. Botulinum toxin (Botox, 50 U per fat pad) or 0.9% normal saline (control) was injected into the center of each of the 2 fat pads. The electrophysiological effects were evaluated at 1, 2, and 3 weeks (7 to 8 animals at each time point) with and without cervical vagal stimulation. The vagal stimulation effects on the sinus and atrioventricular nodes were inhibited, and dispersion of atrial effective refractory period was lower at 1 week in the Botox group. Significant suppression of AF inducibility was observed at 1 week but disappeared at 2 and 3 weeks. These changes were not observed in the control group.

Conclusions—Temporary suppression of vagally mediated AF, for at least 1 week, was achieved with botulinum toxin injection in this canine model. This effect might be associated with reduced dispersion of effective refractory period. A temporary autonomic block using botulinum toxin might be a novel therapeutic option for several clinical conditions such as post–cardiac surgery AF. (Circ Arrhythm Electrophysiol. 2011;4:560-565.)

Key Words: botulinum toxin ■ autonomic nerve ■ atrial fibrillation

The autonomic innervation of the heart is abundant and asymmetrical.1 The postganglionic parasympathetic neurons are primarily located in the fat pads. The postganglionic sympathetic nerves originate from extracardiac sites, the stellate ganglia, and the sympathetic trunks, and they course along the great arteries. Canine hearts have 3 major epicardial fat pads that contain cardiac ganglionated plexi (GP) of the autonomic nervous system.2 In the human heart, the existence and location of epicardial fat pads are similar to those of the canine heart.3,4

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Both parasympathetic and sympathetic stimulation can shorten the atrial effective refractory period (ERP), action potential duration, and reentrant wavelength. Parasympathetic activation also affects the heterogeneity of refractoriness. The initiation and maintenance of atrial fibrillation (AF) are highly dependent on these electrophysiological characteristics; therefore, they might be important in AF pathophysiology. Autonomic denervation effects on AF have been introduced and studied with the use of animal models. AF was not induced after major epicardial fat pad ablation.2 Fat pad ablation was achieved by the transvenous approach to suppress AF.5 In humans, GP ablation combined with pulmonary vein (PV) isolation has been reported to produce favorable results.6–8 However, GP ablation may not have long-term effects on AF suppression.9

Botulinum toxin is a neurotoxin produced by Clostridium botulinum. It has a zinc-binding metalloendopeptidase function. The mechanism of action is the blocking of the exocytotic release of acetylcholine stored in synaptic vesicles.10 Therefore, botulinum toxin blocks cholinergic neurotransmission, which is important in postganglionic neurons. The blocking effects are temporary and are recovered in 1 to 6 months, depending on the injection site.11 Reports on the cardiac effects have not been well established. Injection of botulinum toxin into the sinus node fat pad blocks bradycardia mediated by parasympathetic activation in the dog heart.12 A previous report from the authors’ laboratory demonstrated that the vagal stimulation effects on the sinus node and AF inducibility could be blocked for a few hours after botulinum toxin injection during acute experiments.13 Because of the difficulties in achieving long-term effects after destruction (ablation) of GP structures, the benefits of
such a strategy for antiarrhythmic therapy remain doubtful. However, if the goal is to achieve temporary suppression of AF, as in the post–cardiac surgery scenario, then temporary ganglionic block without destruction of the anatomic structures becomes an appealing possibility. Therefore, we performed this study to assess the short- to mid-term effects of botulinum toxin on AF inducibility.

Methods

Surgical Preparation
The study protocol was approved by Seoul National University Hospital Institutional Animal Care and Use Committee. Twenty-three adult male mongrel dogs, weighing 20 to 25 kg, were studied. Following standard and approved protocols, all dogs were anesthetized with thiopental (20 mg/kg IV), intubated, respirated, and monitored under gaseous anesthesia (1% to 2% isoflurane/O2). All measures were taken to ensure that discomfort, distress, pain, and injury were limited to that which was unavoidable. Standard surface ECG leads were monitored continuously throughout the entire study. Intermittent arterial blood gas measurements were obtained and ventilator adjustments were made to correct for any metabolic abnormalities. An electric heating pad was used to maintain the body temperature of 36° to 37°C.

Vagal Stimulation Protocol
Right cervical vagal stimulation (VS) was applied by using stainless steel electrodes (Model 6491, Unipolar Pediatric Temporary Pacing Lead, Medtronic, Minneapolis, MN). Typical parameters for the cervical VS trains were a frequency of 20 Hz and pulse width of 0.2 ms, with a Grass stimulator (S-88, Astro-Med, Warwick, MA). Because AF inducibility during VS is strength-dependent, the choice of VS intensity is important. On the basis of our previous data,14 the stimulation current amplitude has been fixed at 5.0 V, enough to prolong the spontaneous sinus cycle length at least twice. Data were obtained 5 seconds after the onset of VS, and the typical duration of continuous VS was ≤45 seconds to avoid “fading” effects.

Botulinum Toxin Injection
A right lateral thoracotomy was performed. After exposure of the right posterior side of the heart, botulinum toxin (Allergan, Inc, Irvine, CA; 50 U/1 mL at each fat pad, 14 dogs, Botox) or 0.9% normal saline (1 mL at each fat pad, 9 dogs, control) was injected into the entire visible area of the 2 major epicardial fat pads: the sinus node (right) fat pad and the atroventricular (AV) node (inferior vena cava–left atrium) fat pad. The locations of both fat pads are illustrated in one of the authors’ previous reports.9 The needle tip was positioned manually at several points on the epicardial surface of the fat pads under direct visual control to ensure optimal injection.

Electrophysiological Study Protocol
The following study protocol was applied at 1, 2, and 3 weeks after injection (7 to 8 animals at each time point, n=4 to 5 and n=3 in Botox and control, respectively, Figure 1). We did not evaluate longer term, >3 weeks, because even fat pad destruction by radiofrequency ablation lost its denervation effects at 4 weeks.9 ECG and atrial electrograms were amplified and filtered from 0.05 to 500 Hz and were displayed and recorded on a Prucka Cardiolab EP System (GE Medical Systems, Fairfield, CT). Epicardial pacing electrodes (Capsure Epi, Medtronic, Minneapolis, MN) were placed at the right and left atrial free walls. Atrial pacing was then performed with twice the current threshold. The AV nodal function was evaluated by measuring the ventricular response rate during rapid atrial pacing (VR) with a 50-ms cycle length, mimicking the ventricular response during AF. The sinus rate (SR) and VR were calculated using the average R-R interval of 20 beats. The pacing protocol for the atrial ERP measurement was as follows. The basal pacing cycle length (S1–S1) was 400 ms. The S1–S2 interval was started at 200 ms and decreased by 5-ms steps until the atrial capture

Results

Effects of Botulinum Toxin on the Sinus and AV Nodes
VS slowed SR and VR significantly in both the control and Botox groups (Figures 2 and 3). The averaged data (Figure 2G) indicated that VS effects on SR were as follows: 75±4.3% versus 60±8.0% (P=0.036), 63±9.3% versus 52±7.6% (P=0.18), and 67±9.4% versus 54±3.2% (P=0.11) at 1 week, 2 weeks, and 3 weeks, respectively (Botox versus control, Figure 4A). The effects of VS on VR were as follows: 72±11% versus 46±8.6% (P=0.071), 62±23% versus 35±9.6% (P=0.25), and 43±14% versus 40±15% (P=0.86) at 1 week, 2 weeks, and 3 weeks, respectively (Botox versus control, Figure 3G).
Effects on Atrial ERP and Dispersion of ERP
Both in control dogs and in dogs after Botox injection, VS shortened ERP in both atria significantly at all time points (Figure 4).

ERP of the right atrium (RERP, Figure 4 mol/L) in the Botox group measured during VS was slightly higher than that of the control group, even though statistical significance had not been reached. However, the dispersion of ERP (LERP−RERP) during VS (P=0.048, Figure 5A) was significantly lower at 1 week in the Botox group.

Effects on AF Inducibility
Suppression of AF inducibility was observed at 1 week in the Botox group; however, it disappeared at 2 and 3 weeks: 20±11% versus 58±14% (P=0.025), 30±21% versus 58±14% (P=0.11), and 56±13% versus 67±14% (P=0.41) at 1 week, 2 weeks, and 3 weeks, respectively (Botox versus control, Figure 5B).

Discussion

Major Findings
The goal of this study was to evaluate the short- to mid-term effects of GP block by botulinum toxin on AF inducibility in the dog. The VS effects on dispersion of ERP and AF inducibility were significantly attenuated at 1 week after botulinum toxin injection and recovered at 2 to 3 weeks. These findings suggest that temporary suppression of AF may be achieved with botulinum toxin in a canine model; this might have been associated with reduced dispersion of ERP.

Atrial ERP and Dispersion of ERP
Atrial ERP and dispersion of ERP are associated with AF vulnerability. Parasympathetic activation shortens ERP and increases dispersion of ERP. These changes enhance inducibility and favor maintenance of AF. However, dispersion of ERP may be more important than ERP in vagally mediated AF. For the process of AF initiation, both ERP shortening and
wide dispersion of refractoriness can evoke conduction block or delay to induce AF. For the maintenance of AF, dispersion could play a more important role than ERP per se because a predictor of maintenance of cholinergic AF would be a gradient of action potential duration.15

In the present study, the difference in the effects of botulinum toxin on ERP was not significant in the 2 study groups. Reduced dispersion of ERP was observed at 1 week, in which AF inducibility was suppressed. In a previous animal study that evaluated AF vulnerability after a “single” fat pad ablation, partial denervation increased AF inducibility.16 The investigators observed that ERP was not significantly changed; however, its standard deviation was increased after partial denervation. Therefore, they concluded that dispersion of refractoriness had a more significant role in the ability of premature beats to induce AF than the regional refractoriness near the pacing site in their animal model. In the clinical setting, inducibility of AF during electrophysiological evaluation was associated with increased dispersion of ERP.17 These findings support the results of the present study.

Parasympathetic innervation of the atria is not homogeneous; therefore, its effect on ERP is not homogeneous. The VS might provide stronger effects on a specific region of one of the atria than the other regions of the atria. Therefore, a difference in dispersion of ERP could be more prominent than the ERP. A GP block could attenuate this effect and thereby reduce AF vulnerability.

Cardiac Parasympathetic Autonomic Modification by Ganglionic Block

The epicardial fat pads contain entities representing both branches of the cardiac autonomic nervous system. Nevertheless, a majority of cardiac neurons in the fat pads were found to be cholinergic.18 Although vagal synaptic transmission
within the fat pads is well recognized, the presence of sympathetic neurons and/or bypassing axons is less clear. However, the functional role of the parasympathetic components in the epicardial ganglia is clearly dominant. Electric fat pad stimulation always evokes strong negative chronotropic and dromotropic effects. Therefore, the GP block achieved by Botox injection in the present study reflects parasympathetic effects.

Permanent GP block, for example, with the use of radiofrequency ablation of cardiac fat pads, might be difficult to achieve. This has been previously demonstrated in an animal model.\textsuperscript{9} In addition, several investigators have evaluated human GP ablation as a stand-alone therapy for the treatment of patients with AF. They found that the efficacy and outcome of “GP ablation alone” was not as good as conventional PV isolation procedures, and the AF recurrence rate of GP ablation alone was 71\% to 74\% at 8 to 12 months.\textsuperscript{19,20} However, GP ablation as an adjunctive therapy could be an option for AF ablation. GP ablation in addition to conventional PV isolation has demonstrated better outcomes than PV isolation alone in a randomized clinical trial as well as an observational study and a single-arm study.\textsuperscript{5,8,21}

\section*{Clinical Implications}

Although the mechanism(s) responsible for post–cardiac surgery AF is not well understood, the autonomic imbalance, particularly parasympathetic enhancement, appears to play a role. Postoperative AF typically develops within 1 week. Therefore, temporary block of GP transmission during that time frame would be expected to produce favorable results.

One of the obvious advantages of such a strategy is the nondestructive procedure that has transient but still lasting effects. Destruction of a major GP may not be the first choice, because the long-term effects of removing such important functional structures in humans is not known. Furthermore, permanent GP block may be impossible to achieve. Therefore, temporary autonomic blockade without permanent destruction of the GP, using botulinum toxin, might be a novel alternative therapy.

Other possible therapeutic targets would be situations associated with transient autonomic dysfunction such as neurocardiogenic syncope. Pachon et al\textsuperscript{22} reported radiofrequency GP ablation for patients with this disease entity and its outcomes. Although this type of approach should be evaluated by other trials, a majority of patients with neurocardiogenic syncope usually improve several months or years later; thus, temporary block rather than permanent destruction would be better option for this disease.

\section*{Study Limitations}

Botulinum toxin was not injected into all existing epicardial GPs in the present study. However, the goal was not to accomplish a complete autonomic blockade, though the latter might be difficult to achieve. This is because efferent fibers bypassing the GP have been identified.\textsuperscript{2} Similarly, the modest effects observed in this study might reflect the rather low doses of botulinum toxin. The botulinum toxin applications used were at doses similar to those used for cosmetic procedures; higher botulinum toxin doses might be better suited for cardiac arrhythmia applications.

In addition, the cervical vagal trunks were not decentralized; therefore, the persistence of afferent nerve activation cannot be ruled out. Furthermore, the sympathetic tone was not suppressed, for example, by \(\beta\)-blockers or sympathetic ganglia destruction. Thus, sympathetic activation during the procedures might have affected the results of the present study. However, all vital signs were kept stable in the animals during the experimental procedures. Therefore, the variation of sympathetic tone probably was minimal. Only 1 site in each atrium was evaluated. However, the trend is not likely to be significantly different with a multiple-site evaluation.

Finally, suppression of AF inducibility in this specific animal model does not indicate suppression of AF per se, because noninducibility of AF has limitations in assessing successful catheter ablation. Furthermore, this was an animal study; thus, the procedures used in the dog model must be evaluated for safety before future studies are considered in human patients.

\section*{Conclusion}

The VS effects on AF inducibility were significantly eliminated at 1 week after botulinum toxin injection and recovered at 2 to 3 weeks. These findings suggest that temporary suppression of AF inducibility was achieved with botulinum toxin in this canine model, and this might be associated with reduced dispersion of ERP.

\section*{Sources of Funding}

This study was supported by a grant from the Innovative Research Institute for Cell Therapy (A062260), Republic of Korea.

\section*{Disclosures}

None.

\section*{References}


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Although the mechanism(s) responsible for post–cardiac surgery atrial fibrillation (AF) is not well understood, the autonomic imbalance, particularly parasympathetic enhancement, appears to play a role. Postoperative AF typically develops within 1 week. Therefore, temporary block of ganglionated plexi (GP) transmission during that time frame would be expected to produce favorable results. One of the obvious advantages of GP block with the use of botulinum toxin is the nondestructive procedure that has transient but still lasting effects. Destruction of a major GP may not be the first choice, because the long-term effects of removing such important functional structures in humans is not known. Furthermore, permanent GP block may be impossible to achieve. Therefore, temporary autonomic blockade without permanent destruction of the GP with botulinum toxin might be a novel alternative therapy. Suppression of AF inducibility does not indicate suppression of AF per se because noninducibility of AF has limitations in assessing successful catheter ablation. Future clinical trials of patients after cardiac surgery with the primary end point of AF occurrence can prove the present hypothesis.
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_Circ Arrhythm Electrophysiol_. 2011;4:560-565; originally published online June 9, 2011; doi: 10.1161/CIRCEP.111.961854
_Circulation: Arrhythmia and Electrophysiology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3149. Online ISSN: 1941-3084

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