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A brain-stellate ganglion-atrium network regulates atrial fibrillation vulnerability through macrophages in acute stroke

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ABSTRACT

Aims: New-onset atrial fibrillation (AF) is frequently observed following acute stroke. The aim of this study was to investigate the effects of the brain-stellate ganglion-atrium network on AF vulnerability in a canine model with acute middle cerebral artery occlusion (MCAO).

Materials and methods: Twenty-six dogs were randomly divided into the sham-operated group (n = 6), acute stroke (AS) group (n = 7), stellate ganglion ablation (SGA) group (n = 6) and clodronate liposome (CL) group (n = 7). In the sham-operated group, dogs received craniotomy without MCAO. Cerebral ischemic model was established in AS dogs by right MCAO. Right MCAO along with SGA and CL injection into the atrium was performed in SGA and CL dogs, respectively. After 3 days, atrial electrophysiology, neural activity, and the phenotype and function of macrophages in the atrium were studied in all the dogs.

Key findings: Higher AF inducibility (24.4 ± 4.4% versus 4.4 ± 2.2%, $P < 0.05$) and AF duration (15.7 ± 3.8 s versus 2.6 ± 1.1 s, $P < 0.05$) were observed in the AS group compared with the sham-operated group, and were associated with increased left stellate ganglion activity, higher macrophage infiltration and higher levels of inflammatory cytokines in the atrium. SGA or CL injection sharply suppressed AF inducibility (5.5 ± 2.7% versus 24.4 ± 4.4%; 5.3 ± 3.2% versus 24.4 ± 4.4%, both $P < 0.05$) and AF duration (2.9 ± 1.2 s versus 15.7 ± 3.8 s; 3.6 ± 1.0 s versus 15.7 ± 3.8 s, both $P < 0.05$) in canines with acute stroke.

Significance: A brain-stellate ganglion-atrium network may increase AF vulnerability through macrophage activation after acute stroke.

1. Introduction

It is well known that atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and is considered a key risk factor for ischemic stroke. However, AF can also be the major complication of acute stroke and in turn contribute to stroke recurrence. Previous studies have reported a significantly higher incidence of newly detected arrhythmias in patients with stroke than those without stroke, with AF being the most common [1,2]. The mechanism of poststroke AF remains incompletely understood, although atrial electrical remodeling and structural remodeling are known to contribute to the mechanism of AF occurrence and maintenance [3]. Accumulating evidence has shown that cardiac autonomic activity plays a crucial role in the mechanism of AF. Early studies have shown that the increased sympathetic innervation in the atrium is associated with an increased incidence and duration of AF [4], while follow-up studies have found that some methods of neuromodulation such as, stellate ganglion ablation (SGA), can obviously inhibit AF inducibility by attenuating sympathetic activity [5]. Interestingly, autonomic dysfunction, which is generally evidenced by the dysregulation of heart rate and blood pressure, is common after acute stroke. In a recent study, almost 60% of patients with acute stroke presented a higher low frequency (LF)/high frequency (HF) ratio of

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heart rate variability (HRV), representing an increased sympathetic activity [6].

Norepinephrine (NE), the major neurotransmitter released from postganglionic sympathetic nerve terminals, is known to be a potential pro-inflammatory mediator and can induce the release of multiple inflammatory factors [7]. A recent study found that NE is capable of inducing interleukin-6 (IL-6) production in macrophages via the β-adrenoreceptor-NAD(P)H oxidase system-NF-κB signaling pathway [8]. It has been reported that the infiltration of macrophages increases in atrial tissue of patients with AF [9]. This evidence possibly indicates that the interplay of cardiac autonomic nerve and macrophage functional activation plays a role in the pathophysiology of AF.

Accordingly, we proposed a hypothesis that sympathetic overactivity, as a consequence of acute stroke, can increase the vulnerability to AF by regulating macrophage activity in the atrium. SG is an important neural pathway connecting sympathetic nerves to cardiac nerves. In the present study, we investigated the effects of the brain-SG-atrium network on AF vulnerability and explored the brain-heart network in a canine model of acute stroke.

2. Material and methods

This study was approved by the animal studies subcommittee of our institutional review board and was in compliance with the guidelines of the National Institutes of Health for the care and use of laboratory animals.

2.1. Animal model preparation

Twenty-six beagle canines (weighing 6 to 9 kg) were used in this study. An intramuscular injection of 25 mg/kg ketamine sulfate was administered before pentobarbital sodium (ASPEN Biotechnology Co., Ltd, China) premedication. All of the dogs were premedicated with sodium pentobarbital (30 mg/kg, IV), intubated, and ventilated with room air supplemented with oxygen from a respirator (MAO01746, Harvard Apparatus Holliston, USA). Continuous ECG monitoring was performed. The dogs were randomly assigned to four groups. The sham-operated group consisted of six dogs that underwent craniotomy without right middle cerebral artery occlusion (MCAO). The acute stroke (AS) group consisted of seven dogs in which a cerebral ischemic model was established through the occlusion of the right middle cerebral artery. The SGA group consisted of six dogs that underwent ablation of the left stellate ganglion (LSG) as soon as MCAO was completed. The clodronate liposome (CL) group consisted of seven dogs that underwent unilateral thoracotomy and received CL injection into multiple sites of the atrium including the ganglion plexus (GPs) as soon as MCAO was completed. The dogs in the other three groups besides the CL group underwent unilateral thoracotomy and received saline injection into the atrial myocardium. The detailed experimental design is shown in Fig. 1A.

2.2. Canine model of MCAO

An approximately 4-cm straight incision was made in the vertical zygomatic arch. The skin and subcutaneous layers were cut in turn. The mastoid was used to open the muscles and skin, exposing the temporal bone. The surface of the temporal bone was carefully grinded using an electric drill and then the bone was removed with a needle to form a small bone window of 1-1.5 cm. After a cruciform incision was made in the dura mater performed, the trunk of the right middle cerebral artery (RMCA) was exposed under a microscope. The trunk of the RMCA was electrocoagulated by bipolar electrocoagulation and then cut off. Finally, the wound was sutured in layers. Magnetic resonance imaging (MRI) of the head was performed 24 h after the operation to ensure the success of the stroke model. The MRI images are shown in Fig. 1B.

2.3. LSG ablation

The LSG was completely exposed by a left thoracotomy through the second intercostal space. A radiofrequency current (30–35 W, 150 s) was delivered to the site by an electrode catheter (Biosense Webster, Inc, Diamond Bar, CA) showing blood pressure (BP) elevation during stimulation. Complete ablation was verified by the abolishment of BP elevation during the delivery of electrical stimulation to the ablated site.

2.4. Macrophage depletion in atrial myocardium

After MCAO was completed, thoracotomy and pericardiotomy were performed and the atrium was fully exposed. Macrophage depletion was performed by CL injection into a total of 10 sites of the atrial myocardium (the upper, middle and lower sites of the left and right atria) including the GPs (the right anterior ganglion plexus near the caudal end of the sinoatrial node, the right inferior ganglion plexus at the junction of inferior vena cava and atrium, the left superior ganglion plexus near the junction of left superior pulmonary vein and left pulmonary artery and the left inferior ganglion plexus near the junction of left inferior pulmonary vein and atrium). The upper injection site of the atrium was 5 mm away from the atrial superior border. The middle and lower injection sites were successively spaced 5 mm apart. The effect of macrophage depletion was assessed by flow cytometry. CL was purchased from Vrije Universiteit Amsterdam.

2.5. Electrophysiological measurements

Multielectrode catheters were sutured to the four pulmonary veins and the left and right atria. All recordings were displayed on a computerized electrophysiology system (Lead 7000, Jinjiang Inc., China). The atrial effective refractory period (AERP) and the dispersion of the AERP (dAERP) were measured as previously described [10]. An S1S1 programmed stimulus method (120-ms, 100-ms, and 60-ms cycle length, 5 s each, performed in triplicate for each frequency) was used to assess the inducibility and duration of AF. AF was defined as an irregular atrial rate > 500 bpm lasting for more than 5 s.

2.6. Recordings of neural activity

Autonomic neural activity was measured in the sham-operated group, the AS group and the CL group after 3 days. To avoid the disturbance of electrical stimulation, the recordings of autonomic neural activity were performed before the electrophysiological measurements. Pairs of bipolar hook electrodes (Xi'an Friendship Medical Electronics Co., Ltd., China) were attached to the LSG. All measured signals were amplified and filtered between 0.3 Hz and 1 kHz with a PowerLab system (AD Instruments, Dunedin, New Zealand). Neural signals that were three times higher than noise signal were marked. Neural activity was represented by the frequency (per minute) and amplitude of neural discharges [11].

2.7. Flow cytometry

Left and right atrial tissues were cut into pieces smaller than 5 mm × 5 mm and then minced finely and digested with shaking for 1 h at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing collagenase II (1 mg/ml) (Sigma-Aldrich Co., USA) and DNase I (100 ng/ml) (Sigma-Aldrich Co., USA). During the digestion, the cells in the suspension were dissociated by sequentially passing the extract through 20-, 21- and 23-gauge needles at 30-min intervals. After the digestion, the cells were further dissociated by passing them through a 23-gauge needle three times and filtered through a 40-μm filter. The cells were then centrifuged (400 g for 5 min at 4°C), washed with PBS and resuspended in FACS buffer (PBS + 0.5%BSA + 2mMEDTA).

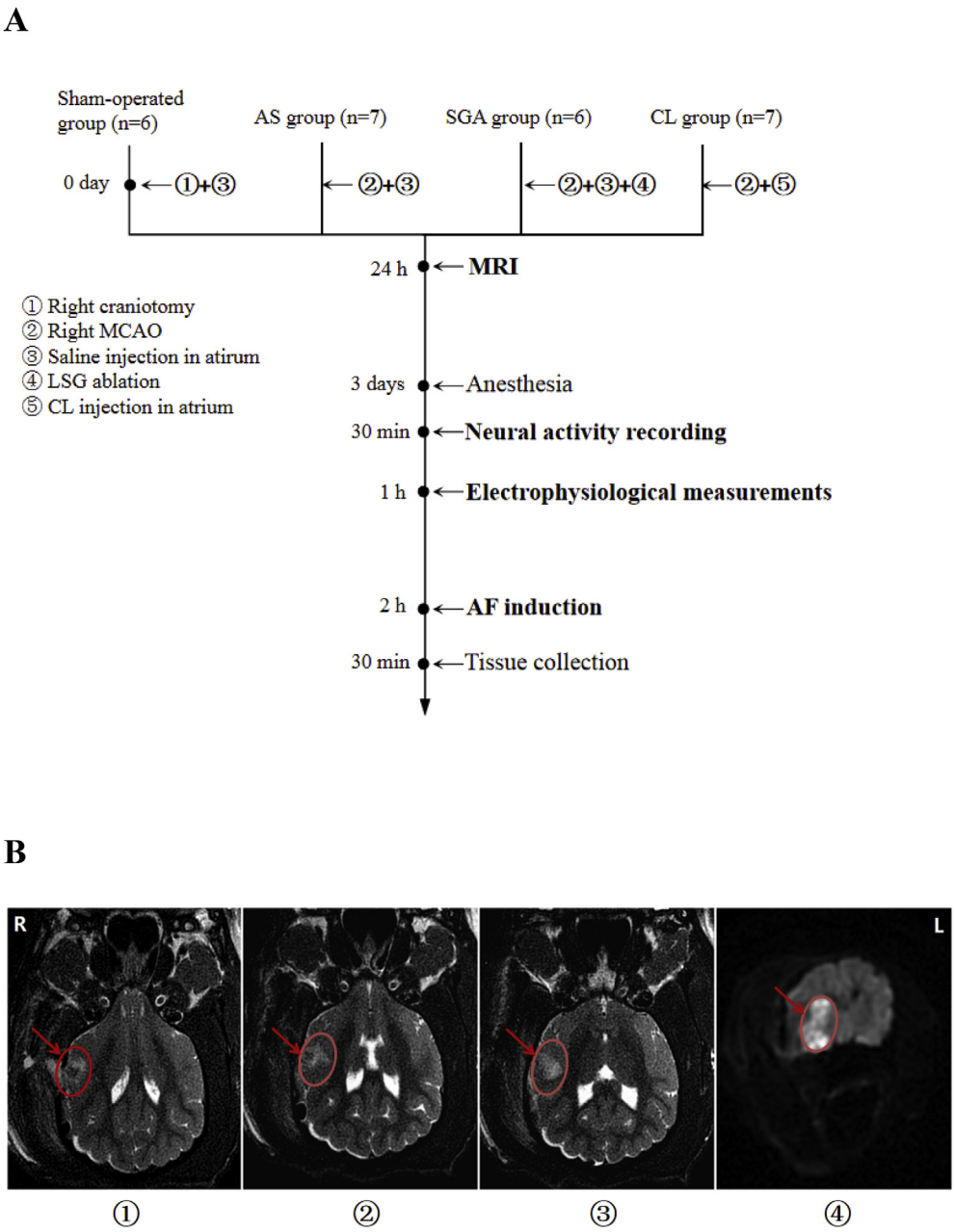

Fig. 1. (A) Experimental design flowchart. (B) A head MRI that was performed 24 h after MCAO suggested the right temporal lobe infarction, marked by arrows and circles. ①-③ Continuous MRI plain scan of the infarcted areas in T2WI. ④ Coronal MRI scan of the infarcted areas in DWI.
Abbreviations: AS, acute stroke; SGA, stellate ganglion ablation; CL, clodronate liposome; MCAO, middle cerebral artery occlusion; LSG, left stellate ganglion; MRI, magnetic resonance imaging; AF, atrial fibrillation.

Antibodies against CD45 (BioLegend, Inc., USA), F4/80 (BioLegend, Inc., USA) and Ly6C (BioLegend, Inc., USA) were used in this study. The samples were incubated for 30 min at 4°C with antibodies and washed in FACS buffer. Then, the cells were subjected to flow cytometry analysis and cell sorting on a BD LSR Fortessa analyzer (Becton, Dickinson and Company, USA). CD45-APC (1:200) + F4/80-PE (1:200) was used to label cardiac resident macrophages, and CD45-APC (1:200) + Ly6C-percpcy5.5 (1:200) were used to label monocytes/macrophages.

2.8. ELISA

Tissue specimens that were obtained from the right atrium (RA) and the left atrium (LA) were temporarily stored at −80°C until the assay. Then, samples from all groups were assigned to measure inflammatory cytokine concentrations in the tissues. The levels of TNF-a, IL-6 and IL-1β were examined by enzyme-linked immunosorbent assay (ELISA).

2.9. Western blotting

The membranes were incubated with a primary antibody against NF-κB (rabbit polyclonal anti-NF-κB antibody, Abcam, Inc., UK), TNF-a (rabbit polyclonal anti-TNF-a antibody, Abcam, Inc., UK), IL-6 (rabbit polyclonal anti-IL-6 antibody, Abcam, Inc., UK) and IL-1β (rabbit polyclonal IL-1β antibody, Abcam, Inc., UK). The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline with Tween 20 (TBST) for 1 h and incubated with the primary antibody overnight at 4 °C. They were then washed in TBST three times, incubated with the secondary antibody for 1 h at 37 °C, and imaged using Immun-Star

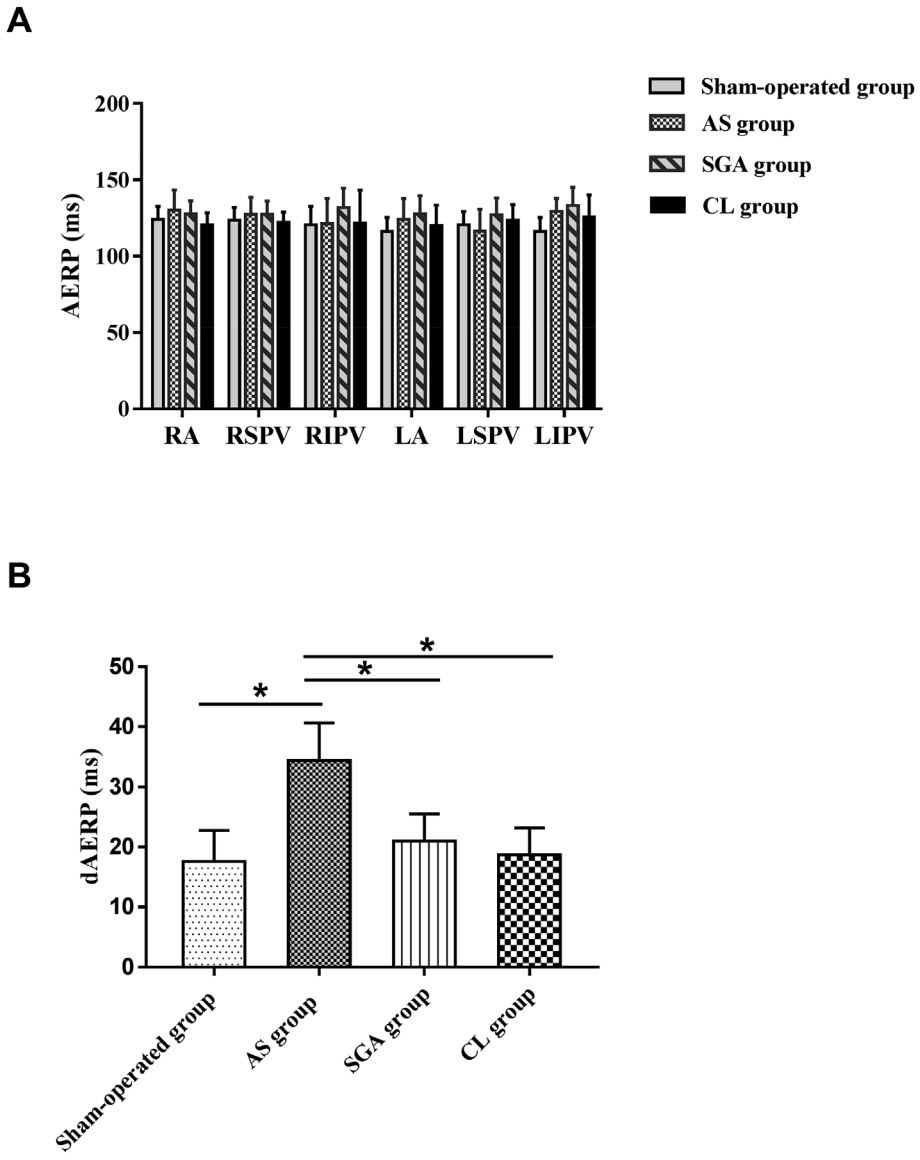

Fig. 2. Differences in the AERP and the dAERP in all groups. (A) There was no significant difference in AERP among the four groups. (B) The AS group had a higher dAERP compared with that in the other three groups, no significant difference was observed among the sham-operated group, the SGA group and the CL group. *$P < 0.05$.
Abbreviations: AERP, atrial effective refractory period; dAERP, dispersion of AERP; AS, acute stroke; SGA, stellate ganglion ablation; CL, clodronate liposome; LA, left atrium; LSPV, left superior pulmonary vein; LIPV, left inferior pulmonary vein; RA, right atrium; RSPV, right superior pulmonary vein; RIPV, right left superior pulmonary vein.

horseradish peroxidase substrate. The relative expression levels of the proteins were determined using image analyzer software (AlphaEase FC, San Leandro, CA, USA).

2.10. Statistical analysis

The data are expressed as the mean ± SEM. Two-sample independent Student's t-tests were used to compare the means of two groups. ANOVA followed by Newman–Keuls tests was used to compare the mean values of continuous variables among multiple groups, and any significant differences were further analyzed using the Tukey-Kramer test. All the statistical tests were two-sided, and a probability value < 0.05 was required for statistical significance.

3. Results

3.1. Electrophysiological testing and AF induction

As shown in Fig. 2, there were no significant differences in the AERP at any of the recording sites among all groups. However, compared with that in the AS group, the dAERP was prominently decreased in the SGA group (21.0 ± 3.6 ms versus 36.5 ± 4.8 ms, P < 0.05) and in the CL group (17.6 ± 2.8 ms versus 36.5 ± 4.8 ms, P < 0.05), while no significant difference was observed among the sham-operated group, AS group and CL group.

As shown in Fig. 3, the mean AF inducibility (24.4 ± 4.4% versus 4.4 ± 2.2%, $P < 0.05$) and AF duration (15.7 ± 3.8 s versus 2.6 ± 1.1 s, $P < 0.05$) were significantly higher in the AS group than in the sham-operated group. Compared with those in the AS group, AF inducibility and AF duration were markedly reduced in the SGA group (inducibility: 5.5 ± 2.7% versus 24.4 ± 4.4%, $P < 0.05$; duration: 2.9 ± 1.2 s versus 15.7 ± 3.8 s, $P < 0.05$) and the CL group (inducibility: 5.3 ± 3.2% versus 24.4 ± 4.4%, $P < 0.05$; duration: 3.6 ± 1.0 s versus 15.7 ± 3.8 s, $P < 0.05$). There were no significant differences in the AF inducibility or AF duration among the sham-operated group, the SGA group and the CL group.

3.2. Recordings of sympathetic activity

The neural activity of the LSG was recorded 3 days after operation. The results are shown in Fig. 4. Compared with those in the sham-operated group, the frequency and amplitude of the LSG were significantly elevated in the AS group (frequency: 59 ± 12 versus 452 ± 39 impulses/min, $P < 0.05$; amplitude: 0.0376 ± 0.002 versus

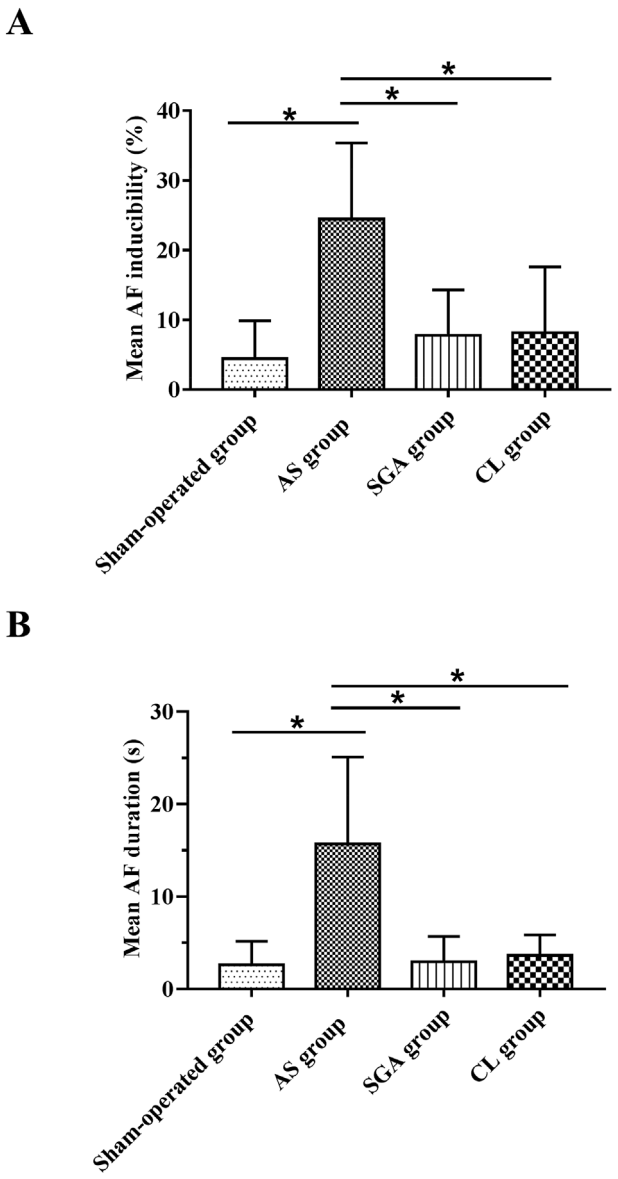

Fig. 3. The mean AF inducibility and AF duration in all groups. (A) The mean AF inducibility ratio in the AS group was greatly higher than that in the other three groups. There was no significant difference in the AF inducibility among the sham-operated group, the SGA group and the CL group. (B) The AS group had a higher AF duration compared with that in the other groups. There was no significant difference in the AF duration among the sham-operated group, the SGA group and the CL group. *$P < 0.05$.

Abbreviations: AF, atrial fibrillation; AS, acute stroke; SGA, stellate ganglion ablation; CL, clodronate liposome.

0.0675 ± 0.004 mV, $P < 0.05$) and the CL group (frequency: 59 ± 12 versus 443 ± 53 impulses/min, $P < 0.05$; amplitude: 0.0376 ± 0.002 versus 0.0632 ± 0.005 mV, $P < 0.05$). There was no significant difference in the neural activity of the LSG between the AS group and the CL group.

3.3. Flow cytometry

Compared with that in the sham-operated group, the concentration of macrophages in the atrial myocardium was significantly higher in the AS group. The ratio of $CD45^+F4/80^{hi}$ macrophages was 3.2 ± 0.5% in the sham-operated group and 8.4 ± 0.7% in the AS group, respectively ($P < 0.05$). Furthermore, the infiltration of $CD45^+Ly6C^{hi}$ monocytes/macrophages was also increased in the AS group when compared with the sham-operated group (5.4 ± 0.3% versus 1.8 ± 0.3%, $P < 0.05$). Compared with that in the AS group, the ratio of both $CD45^+F4/80^{hi}$ macrophages and $CD45^+Ly6C^{hi}$ monocytes/macrophages was significantly reduced in the SGA group (3.4 ± 0.9% versus 8.4 ± 0.7%, $P < 0.05$; 3.0 ± 0.6% versus 5.4 ± 0.3%, $P < 0.05$) and the CL group (4.1 ± 0.6% versus 8.4 ± 0.7%, $P < 0.05$; 2.5 ± 0.4% versus 5.4 ± 0.3%, $P < 0.05$). No significant difference in the concentration of macrophages was observed among the sham-operated group, the SGA group and the CL group (Fig. 5).

3.4. Inflammatory cytokines

The levels of inflammatory cytokines in atrial tissue are shown in Table 1. Compared with those in the sham-operated group, the levels of TNF-α, IL-6 and IL-1β in the RA and LA were greatly increased in the AS group (RA: TNF-α: 449.5 ± 3.8 versus 115.2 ± 4.1 pg/ml; IL-6: 529.5 ± 17.6 versus 226.1 ± 9.1 pg/ml; IL-1β: 198.6 ± 7.6 versus 48.3 ± 2.5 pg/ml; LA: TNF-α: 455.9 ± 8.3 versus 123.2 ± 4.1 pg/ml; IL-6: 466.7 ± 15.6 versus 230.1 ± 5.4 pg/ml; IL-1β: 184.4 ± 4.9 versus 45.5 ± 2.9 pg/ml; $P < 0.05$ for all). The concentration of inflammatory cytokines in atrial tissue was markedly decreased in the SGA group and the CL group compared with the AS group ($P < 0.05$ for all). Compared with those in the sham-operated group and the SGA group, the levels of TNF-α, IL-6 and IL-1β in atrial tissue were higher in the CL group ($P < 0.05$ for all). No significant difference in the concentration of inflammatory cytokines in atrial tissue was observed between the sham-operated group and the SGA group.

3.5. Western blotting studies

All immunoblot band intensity measurements were normalized to the intensity of the GAPDH band in the loaded sample. The expression of NF-κB p65 protein was markedly higher in the AS group than that in the sham-operated group (RA: 0.611 ± 0.06 versus 0.165 ± 0.02, $P < 0.05$; LA: 0.653 ± 0.07 versus 0.109 ± 0.02, $P < 0.05$), in the SGA group (RA: 0.611 ± 0.06 versus 0.176 ± 0.04, $P < 0.05$; LA: 0.113 ± 0.02 versus 0.653 ± 0.07, $P < 0.05$) and in the CL group (RA: 0.611 ± 0.06 versus 0.345 ± 0.05, $P < 0.05$; LA: 0.294 ± 0.04 versus 0.653 ± 0.07, $P < 0.05$). Compared with that in the CL group, the expression of NF-κB was lower in the SGA group (RA: 0.176 ± 0.04 versus 0.345 ± 0.05, $P < 0.05$; LA: 0.113 ± 0.02 versus 0.294 ± 0.04, $P < 0.05$). No significant difference in the level of NF-κB p65 in atrial tissue was observed between the SGA group and the sham-operated group (Fig. 6). Compared with those in the sham-operated group, the levels of TNF-α, IL-6 and IL-1β in the RA and LA were markedly increased in the AS group (RA: TNF-α: 0.317 ± 0.023 versus 0.079 ± 0.006; IL-6: 0.537 ± 0.028 versus 0.125 ± 0.010; IL-1β: 0.727 ± 0.039 versus 0.158 ± 0.014; LA: TNF-α: 0.483 ± 0.026 versus 0.116 ± 0.007; IL-6: 0.519 ± 0.024 versus 0.119 ± 0.016; IL-1β: 0.778 ± 0.039 versus 0.189 ± 0.018; $P < 0.05$ for all). The levels of inflammatory cytokines in the atrial tissue were markedly decreased in the SGA group (RA: TNF-α: 0.089 ± 0.005 versus 0.317 ± 0.023; IL-6: 0.155 ± 0.014 versus 0.537 ± 0.028; IL-1β: 0.197 ± 0.021 versus 0.727 ± 0.039; LA: TNF-α: 0.135 ± 0.009 versus 0.483 ± 0.026; IL-6: 0.132 ± 0.024 versus 0.519 ± 0.024; IL-1β: 0.205 ± 0.021 versus 0.778 ± 0.039; $P < 0.05$ for all) and the CL group (RA: TNF-α: 0.145 ± 0.016 versus 0.317 ± 0.023; IL-6: 0.249 ± 0.023 versus 0.537 ± 0.028; IL-1β: 0.304 ± 0.024 versus 0.727 ± 0.039; LA: TNF-α: 0.224 ± 0.014 versus 0.483 ± 0.026; IL-6: 0.215 ± 0.027 versus 0.519 ± 0.024; IL-1β: 0.316 ± 0.030 versus 0.778 ± 0.039; $P < 0.05$ for all) compared with those in the AS group. Compared with those in the sham-operated group and the SGA group, the levels of TNF-α, IL-6 and IL-1β in atrial tissue were higher in the CL group ($P < 0.05$ for all). No significant difference in the concentration of inflammatory cytokines in atrial tissue was observed between the sham-operated group and the SGA group (Fig. 7).

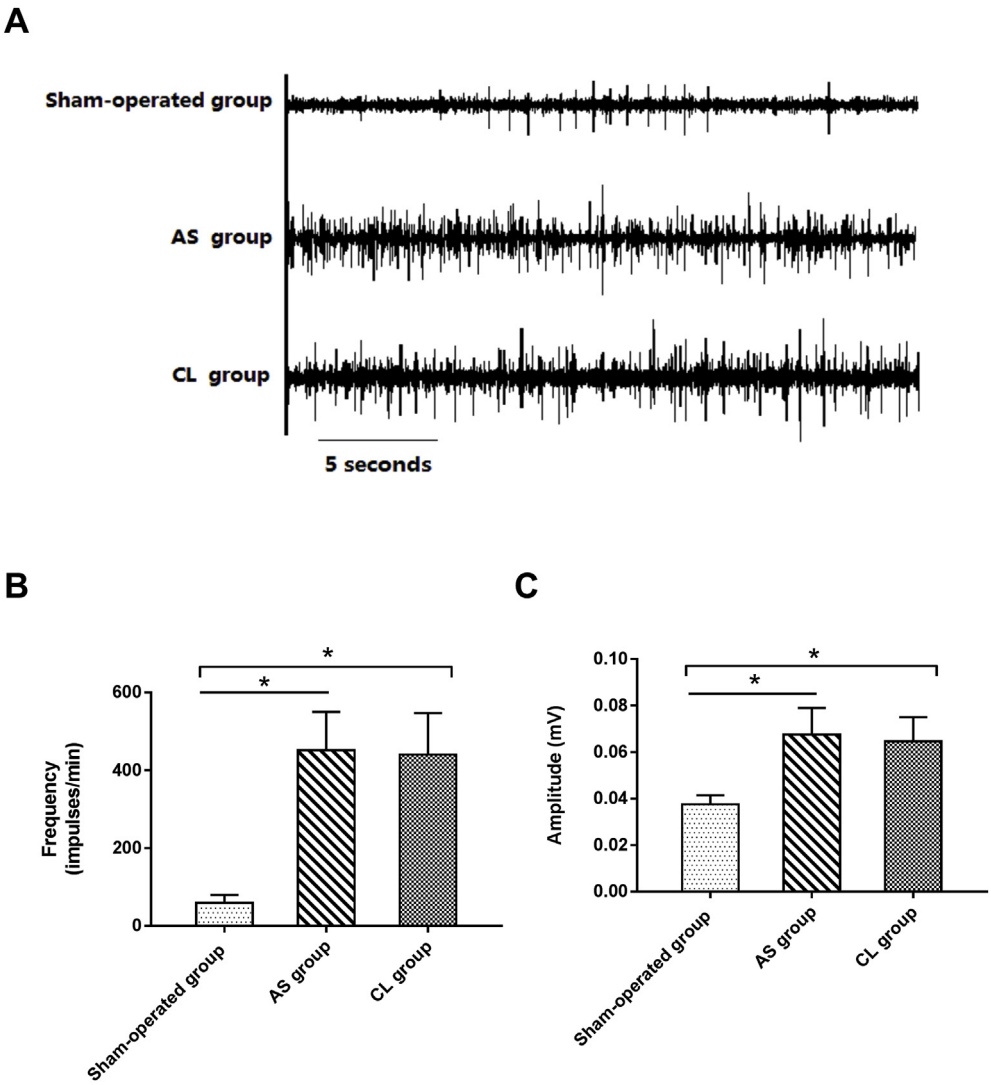

Fig. 4. The neural activity of the LSG in the sham-operated group, the AS group and the CL group. (A) The neural activitiy of the LSG 3 days after craniotomy. (B and C) The frequency and the amplitude in the AS group and the CL group elevated greatly compared with the sham-operated group. There were no significant differences in both frequency or amplitude between the AS group and the CL group. $*P < 0.05$.
Abbreviations: left stellate ganglion; AS, acute stroke; CL, clodronate liposome.

4. Discussion

This study explored the influence of the brain-cardiac network on AF vulnerability in a canine model of MCAO. We provided evidence of the following: (1)increased AF vulnerability, which was associated with LSG hyperactivation, the increased infiltration of macrophages and elevated levels of TNF-α, IL-6 and IL-1β in the atrium, can be observed in canines with acute stroke; (2) SGA or atrial macrophage depletion can both prevent the increased AF vulnerability after an acute stroke; and (3) the effects of SGA on AF vulnerability are associated with macrophage activation and the pro-inflammatory NF-κB signaling pathway.

Previous clinical studies have reported a significantly higher frequency of newly detected arrhythmias, primarily AF, in acute stroke patients [1]. It has been reported that new AF is mostly detected within 3-10 days after acute stroke [12]. In addition, electrocardiographic abnormalities and arrhythmias are more frequently observed in ischemic stroke involving the middle cerebral artery territory [13]. However, animal investigations are lacking, and the underlying mechanism remains incompletely understood. Previous studies have reported a higher LF/HF ratio of HRV in patients with acute ischemic stroke, reflecting greater sympathetic tone relative to parasympathetic tone [6,14]. It is well acknowledged that sympathetic overactivity plays a crucial role in AF occurrence and maintenance. In an animal study, Ogawa et al. found that SGA significantly decreased the incidence of paroxysmal atrial tachycardia and extended sinus pause episodes induced by sympathetic overactivation [5]. In the present study, we found that neural discharges of the LSG and AF inducibility were significantly increased in canines with acute stroke, while LSG ablation significantly prevented the increased AF vulnerability caused by acute ischemic stroke.

Studies have reported higher levels of inflammatory biomarkers in the atrium, as well as increased infiltration of monocytes and macrophages in patients with AF [15]. Inflammatory factors such as TNF-α and IL-6 have been demonstrated to induce atrial electrical remodeling and structural remodeling. For example, TNF-α can decrease sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) expression and lead to structural alterations in the myocardium, such as fibrosis, extracellular matrix degradation, and apoptosis of cardiomyocytes [16]. In contrast, a series of studies revealed that interfering with autonomic nerves or median nerves can prevent AF occurrence and reduce the levels of inflammatory factors [17,18]. A recent study showed that AF can

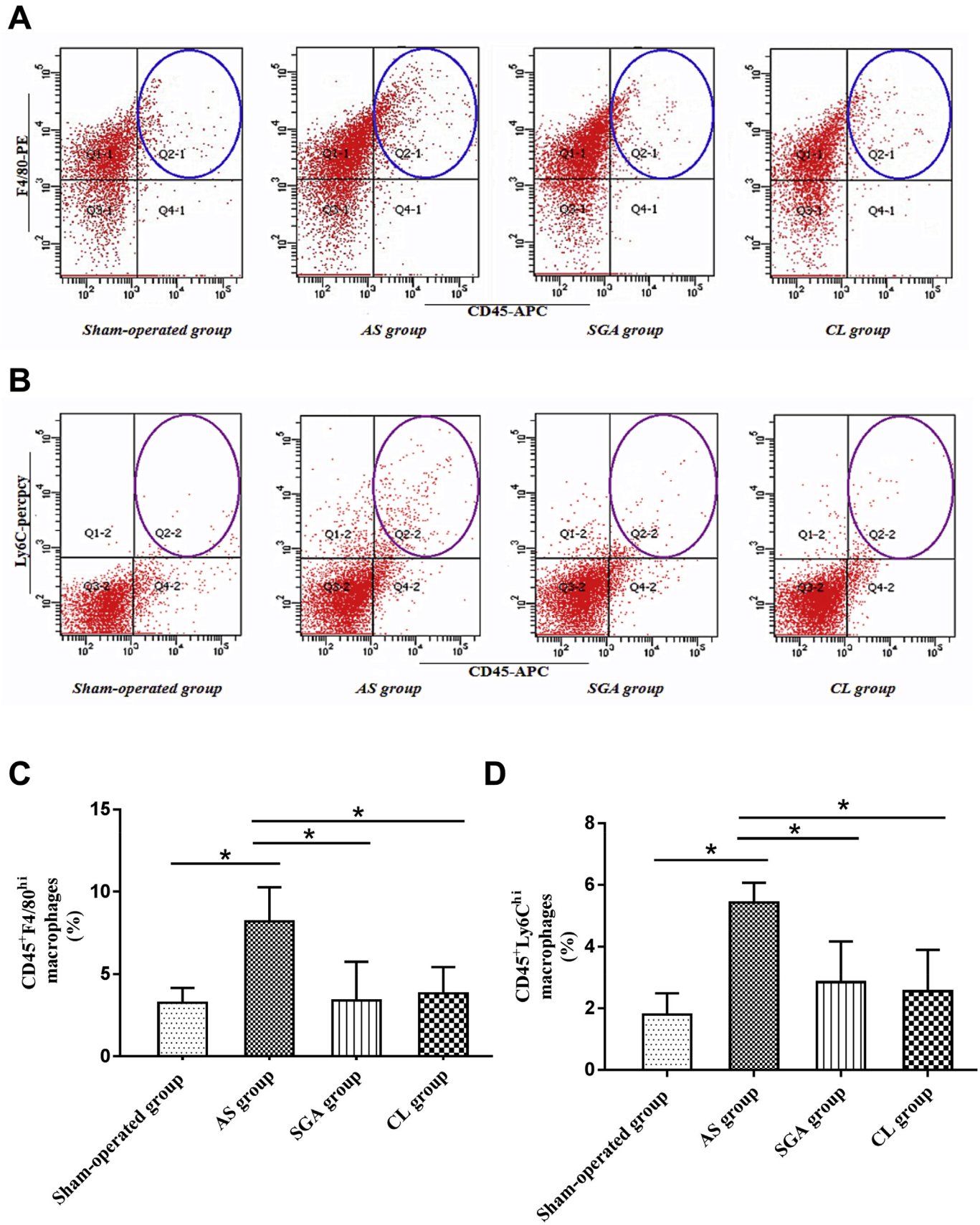

Fig. 5. The macrophage infiltration in atrial tissue in all groups. (A) The flow cytometry of $CD45^+F4/80^{hi}$ macrophage in atrial tissue. (B) The flow cytometry of $CD45^+Ly6C^{hi}$ monocyte/macrophage in atrial tissue. (C and D) The infiltration of both $CD45^+F4/80^{hi}$ macrophage and $CD45^+Ly6C^{hi}$ monocyte/macrophage in atrial tissue in the AS group was higher than that in the other three groups. There was no significant difference in macrophage infiltration among the sham-operated group, the SGA group and the CL group. $^*P < 0.05$.

Abbreviations: AS, acute stroke; SGA, stellate ganglion ablation; CL, clodronate liposome.

Table 1
The levels of inflammatory cytokines in atrial tissue.

	RA			LA		
	TNF-α (pg/ml)	IL-6 (pg/ml)	IL-1β (pg/ml)	TNF-α (pg/ml)	IL-6 (pg/ml)	IL-1β (pg/ml)
Sham-operated group (n = 6)	115.2 ± 4.1*Δ	226.1 ± 9.1*Δ	48.3 ± 2.5*Δ	123.2 ± 4.1*Δ	230.1 ± 5.4*Δ	45.5 ± 2.9*Δ
AS group (n = 7)	449.5 ± 3.8	529.5 ± 17.6	198.6 ± 7.6	455.9 ± 8.3	466.7 ± 15.6	184.4 ± 4.9
SGA group (n = 6)	129.1 ± 7.8*Δ	247.6 ± 9.4*Δ	48.5 ± 2.9*Δ	121.6 ± 2.1*Δ	237.2 ± 4.4*Δ	46.4 ± 2.5*Δ
CL group (n = 7)	172.5 ± 5.2*	309.9 ± 6.9*	73.2 ± 2.6*	179.9 ± 4.0*	314.9 ± 6.5*	66.2 ± 1.6*

$P^{*} < 0.05$ vs the AS group.
$P^{\Delta} < 0.05$ vs the CL group.
Abbreviations: RA, right atrium; LA, left atrium; AS, acute stroke; SGA, stellate ganglion ablation; CL, clodronate liposome; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; IL-1β, interleuki-1 beta.

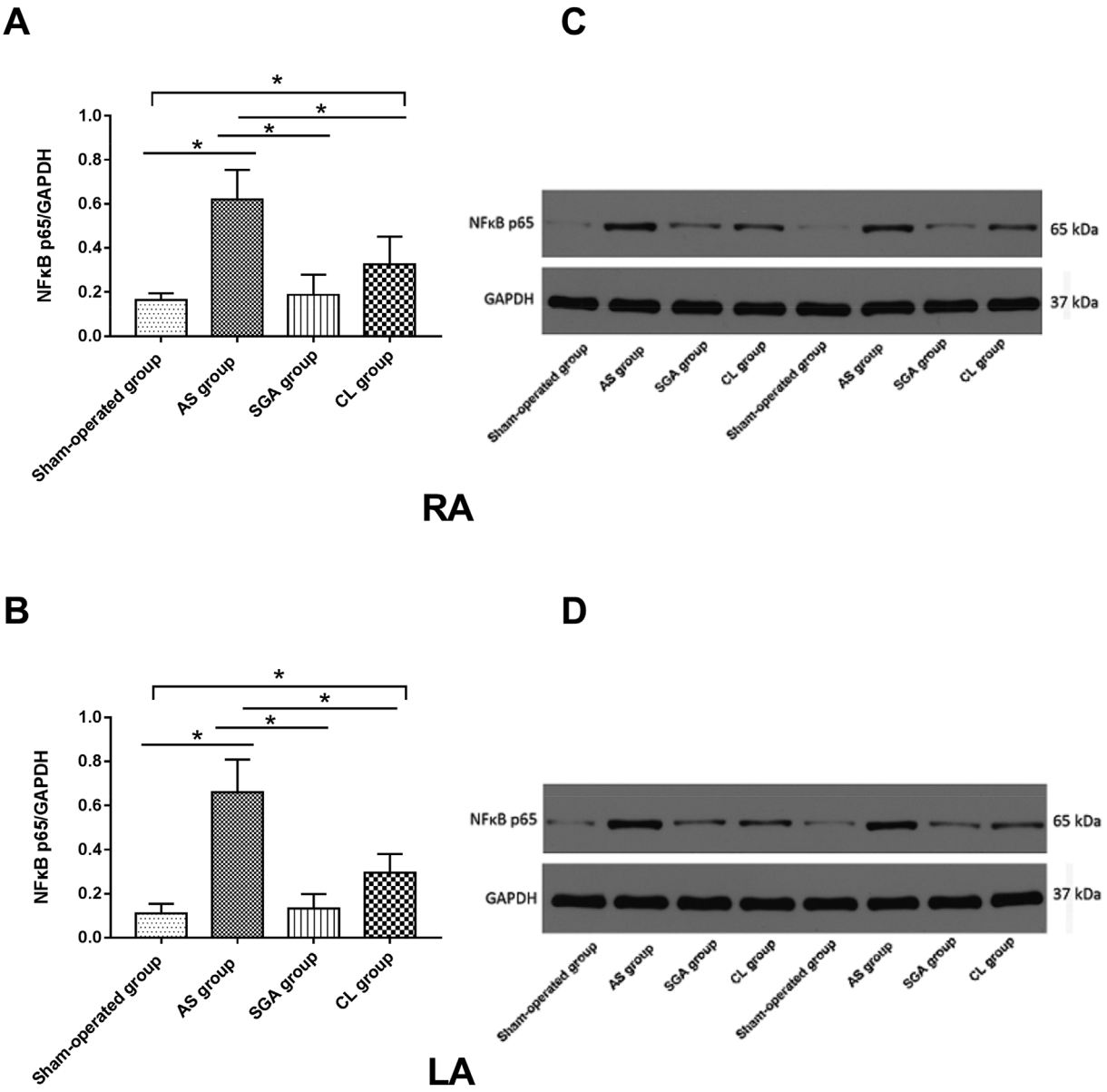

Fig. 6. The expression of NF-κB p65 in RA and LA in all groups. (A and B) The expression of NF-κB p65 in RA and LA in the AS group was greatly higher than that in the other three groups. Compared with that in the CL group, the expression of NF-κB p65 in RA and LA was lower in the SGA group. There was no significant difference in the expression of NF-κB p65 in atrial tissue between the sham-operated group and the SGA group. $*P < 0.05$. (C) The Western blotting of NF-κB p65 in RA. (D) The Western blotting of NF-κB p65 in LA.
Abbreviations: NF-κB, nuclear factor kappa-B; RA, right atrium; LA, left atrium; AS, acute stroke; SGA, stellate ganglion ablation; CL, clodronate liposome.

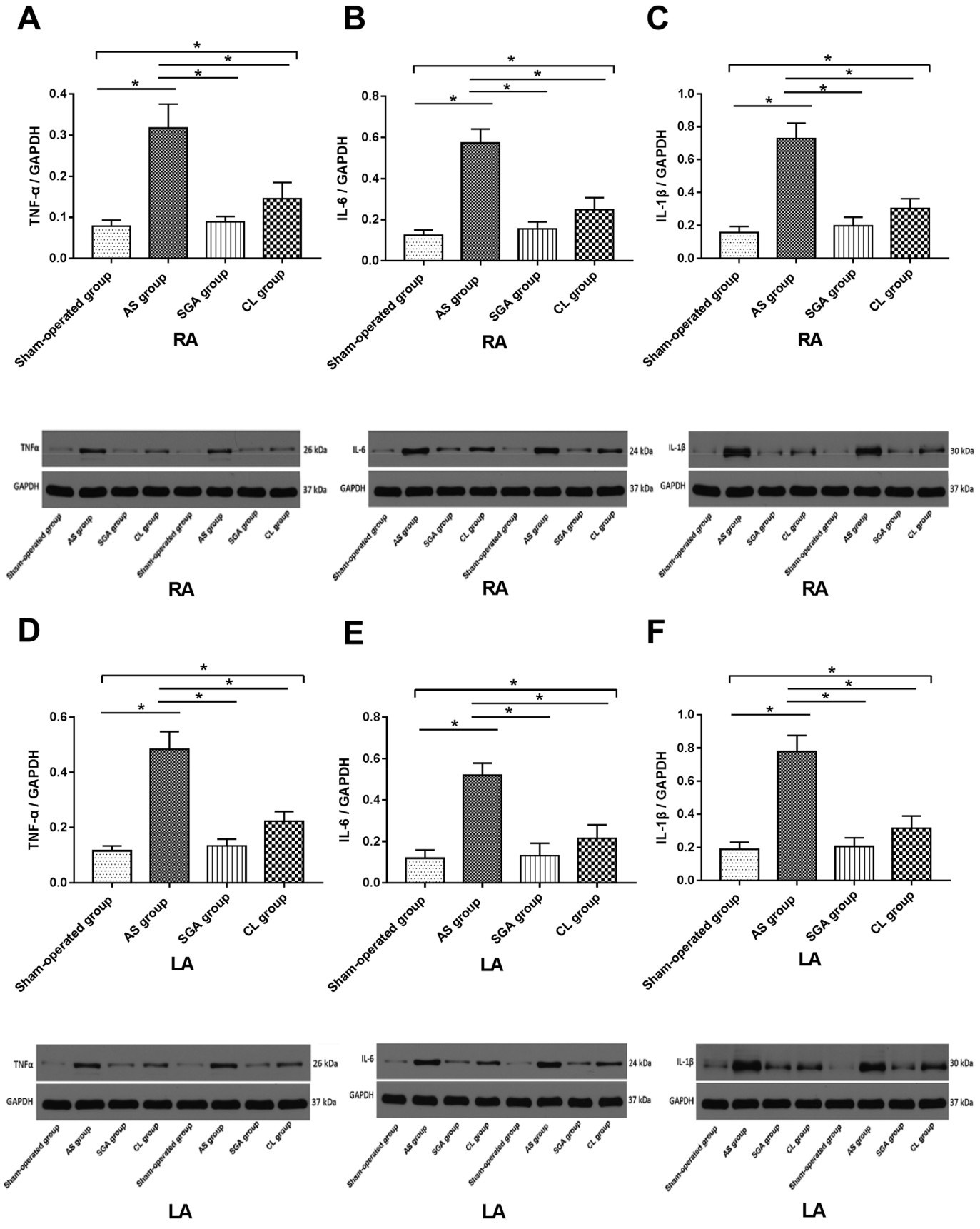

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Fig. 7. The Western blotting results of inflammatory factors in RA (A, B and C) and LA (D, E and F). The expressions of TNF-α, IL-6 and IL-1β in RA and LA in the AS group were greatly higher than those in the other three groups. Compared with those in the CL group, the levels of TNF-α, IL-6 and IL-1β in RA and LA were lower in the SGA group. There were no significant differences in the levels of TNF-α, IL-6 or IL-1β in the atrial tissue between the sham-operated group and the SGA group. *P < 0.05.

Abbreviations: TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; IL-1β, interleukin-1 beta; RA, right atrium; LA, left atrium; AS, acute stroke; SGA, stellate ganglion ablation; CL, clodronate liposome.

promote macrophage polarization to the proinflammatory M1 phenotype, and that proinflammatory macrophages further lead to atrial electrical remodeling. In the acute phase of stroke, activated microglia and macrophages are polarized to the proinflammatory M1 phenotype and release proinflammatory cytokines, such as TNF-α, IL-6 and IL-1β [19]. Increased blood-brain barrier permeability, caused by damaged endothelial cells and further aggravated by proinflammatory cytokines, facilitates the passage of these inflammatory factors into the peripheral circulation, thereby inducing systemic inflammation [20]. However, no previous study has explored the relationship between local cardiac inflammation and acute ischemic stroke. A histopathologic study documented that the infiltration of macrophages was much higher in the myocardium of patients who died after subarachnoid hemorrhage [21]. In the present study, we found that macrophage infiltration in the atrium increased significantly after ischemic stroke. To distinguish different types of cells, macrophages were double labeled with CD45, F4/80 and Ly6C by flow cytometry. We found that the number of $CD45^+F4/80^{hi}$ and $CD45^+Ly6C^{hi}$ cells, representing cardiac resident macrophages and circulating monocyte/macrophages, respectively, was markedly increased. The results indicated that the sources of the increased number of macrophages in the atrium included both circulating monocytes and cardiac resident macrophages after acute stroke. Along with macrophage infiltration, the levels of TNF-α, IL-6 and IL-1β in atrial tissue were increased in canines with acute stroke, while macrophage depletion in the myocardium by CL injection ~~could~~ significantly prevented AF induction and reduced the levels of TNF-α, IL-6 and IL-1β in the atrium. Furthermore, we found that the ablation of LSG also prevented the increased accumulation of macrophages and decreased the levels of inflammatory cytokines in canines with acute stroke. These results showed that the effect of the LSG on inflammatory cytokines was related to macrophage activation.

Previously, NE, a neurotransmitter released from sympathetic fiber endings, was demonstrated to be a potential proinflammatory mediator and to induce the release of multiple inflammatory factors. A recent study reported that NE is capable of inducing IL-6 production in macrophages via the β-adrenoreceptor-NAD(P)H oxidase system-NF-κB signaling pathway. The NF-κB signaling pathway plays an essential role in macrophage-initiated inflammatory responses, promoting proinflammatory macrophage polarization [22]. In the present study, we found that the expression of the p65 subunit of NF-κB in atrial myocytes was elevated after acute stroke and that SGA induced the downregulation of NF-κB p65, which suggested that LSG hyperactivity increased inflammatory cytokine levels in the atrium through the NF-κB signaling pathway after acute stroke.

4.1Study limitations

This study has several limitations. First, in this study, we explored AF vulnerability in the acute phase of stroke, but did not observe spontaneous AF after acute stroke. Previous studies have suggested that a longer duration of monitoring leads to substantially increased detection of AF after ischemic events, and whether the long-term effect of ischemic stroke on AF vulnerability involves the same mechanisms as our present study should be further investigated. Second, we found that increased macrophage infiltration in the atrium involved both circulating monocytes and cardiac resident macrophages after acute stroke, but we failed to further distinguish between proinflammatory and anti-inflammatory types. Given that elevated levels of inflammatory cytokines and NF-κB proteins are associated with increased AF inducibility after acute stroke, we suggest that proinflammatory polarization is predominant in macrophage activation. Finally, we did not explore the effect of different brain lesion locations on AF. Previous studies have suggested the asymmetry of the bilateral insular cortex in the regulation of the autonomic nervous system. However, whether different insular damage has different influences on AF remains unknown.

5. Conclusions

In our study, we demonstrated for the first time that sympathetic activity increases after an acute ischemic stroke, which can further lead to increased AF vulnerability by regulating macrophage activation. SGA or macrophage depletion can prevent macrophage activation and reduce AF vulnerability after acute stroke. Our results support the notion that a brain-SG-atrium network increases AF vulnerability through macrophage activation after acute stroke.

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Declaration of competing interest

None declared.

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