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Bilateral superior cervical ganglionectomy attenuates the progression of β-aminopropionitrile-induced aortic dissection in rats

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ABSTRACT

Aims: Aortic dissection (AD) represents one of the most common aortic emergencies with high incidence of morbidity and mortality. Clinical studies have shown that the increased excitability of the sympathetic nerve may be associated with the formation of AD. In this study, we examined the effects of bilateral superior cervical sympathectomy (SCGx) on the progression of β-aminopropionitrile (BAPN)-induced AD in rats.
Main methods: Sprague–Dawley rats were randomly divided into three groups, including BAPN, BAPN + SCGx and control groups. For terminal measurements, the mean arterial pressure (MAP) and heart rate (HR) were monitored and the basal sympathetic nerve activity (SNA) was assessed through recording the variation in arterial pressure in response to hexamethonium application. Pathological changes in the aortic wall were observed by histological staining. Matrix metalloproteinase-2 (MMP-2) and MMP-9 concentrations within the aortic wall were analyzed by western blot.
Key findings: The results show that BAPN administration could elevate SNA and cause the formation of AD in rats with a high incidence (67.7%), while SCGx treatment inhibited the elevation of SNA and significantly reduced the incidence (20%). SCGx may suppress the formation of BAPN-induced AD via restraining the rise of HR and reducing the MMP-9 concentration in aortic wall.
Significance: These results indicate that surgical techniques such as sympathetic nerve block may be a potentially useful therapy for the prevention of AD.

1. Introduction

Aortic dissection (AD) is considered one of the most lethal events to affect the aorta with an incidence of 3–5 cases per 100,000 people per year [1]. AD is due to a tear in the intimal layer of the aorta or intramural haematoma, which commonly is preceded by medial wall degeneration or cystic media necrosis [2]. Although the exact mechanism of AD formation is still unclear, the progression of AD could be facilitated by a combination of cardiovascular system disorders, such as increased hemodynamic stress, aortic injury and chronic inflammation [3]. The sympathetic nervous system (SNS) is known to play an important role in short and long term regulation of different functions of the cardiovascular system. The SNS influences vascular function through multiple mechanisms, including direct vasoconstriction, wall remodeling, blood pressure increase, and metabolic alterations [4]. Clinical studies have shown that the increased excitability of the sympathetic nerve may be associated with the formation of AD [5]. β-blockers, a kind of adrenergic antagonists, were intensively used in clinical practice for preventing AD and had satisfactory clinical effect [6,7]. Sympathetic ganglion block (SGB) has been reported to attenuate the severity of symptoms and slowed the progression of several cardiovascular diseases including ventricular arrhythmias and pulmonary arterial hypertension [8–10]. SGB is believed to decrease efferent cervical sympathetic outflows and might also inhibit the sympathetic nerve activity (SNA). Considering the effective roles of sympathetic denervation on cardiovascular system disorders, we hypothesized that sympathetic blocks can also attenuate the progression of AD.

β-Aminopropionitrile (BAPN)-treated animals have been widely used as a model of aortic dissecting aneurysm, and the variations in the elastic architecture of the aorta induced by BAPN treatment appear to resemble those found in Marfan's syndrome [11]. Several researches had shown that β-blockers could prevent BAPN-induced aortic ruptures and were effective in the treatment of aortic dissection induced by BAPN [12–14]. Whether the blocking of the sympathetic nerve may affect the formation of BAPN-induced AD has rarely been studied.

In this study, to investigate our hypothesis, we examined the effects of bilateral superior cervical sympathectomy (SCGx) on the progression of BAPN-induced AD in rats.

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2. Material and methods

2.1. Animals and treatments

Experiments were performed on three-week-old male Sprague–Dawley rats weighing 50.1 ± 0.2 g. Forty rats were randomly divided into the following three groups. Group A (n = 15) received BAPN (Sigma-Aldrich, St. Louis, MO) in a dose of 666 mg/kg/day dissolved in saline by subcutaneous injection [15]. The rats in group B (n = 15) received the same injection as group A after the procedure of bilateral SCGx. Group C (n = 10) served as control group. All the rats received three days' rest for acclimatization before the experiment process and postoperative recovery. BAPN was given as one daily injection subcutaneously into the nape of the neck in the morning. The rats were allowed access to standard rat chow and distilled water ad libitum during this experimental period. Body weight was measured once a week. The study lasted four weeks and animals that died during the study were autopsied immediately. Animals that did not die were sacrificed by an overdose of anesthetics at the end of the study. All experimental procedures involving rats were approved by the Experimental Animal Care and Use Committee of Nanjing Medical University and registered under the number IACUC-1705025. All efforts were made to minimize the number of animals used and their suffering.

2.2. Bilateral superior cervical ganglionectomy (SCGx)

SCGx is regarded as a procedure of long-term and repeated SGB. All surgeries were conducted in designated veterinary operating room with sterilized instruments and procedure. For all surgeries, rats were anesthetized with 10% chloral hydrate (0.03 ml/kg, ip) before surgery. A 2-cm incision was performed at the neck region. The common carotid artery was exposed and the superior cervical ganglion was identified behind the carotid bifurcation. Cervical sympathetic nerves running beside the common carotid artery were freed of fat and connective tissues [16]. Both sides of the superior cervical ganglia were cut off and then the incision was closed. Successful sympathetic nerve blockage was confirmed by ptosis of the eyes.

2.3. Mean arterial pressure (MAP) and heart rate (HR) measurements

Mean arterial pressure (MAP) and heart rate (HR) were monitored with a noninvasive computerized tail-cuff system (NIBP; AD Instruments, Australia) in conscious rats. The rats were trained to adapt to the measuring equipment for at least 1 week before experiments. The MAP and HR were calculated by averaging 20 measurements.

2.4. Evaluation of sympathetic nerve activity (SNA)

Each rat was anesthetized with α-chloralose (40 mg/kg ip) and urethane (900 mg/kg ip). A midline incision in the neck was made and the carotid artery was cannulated for measurement of MAP. The femoral vein was also cannulated for drug infusion. Hexamethonium bromide, a nicotinic cholinergic receptor antagonist, was used to assess basal SNA in rats through the variation in MAP in response to the blockage of cervical sympathetic ganglion. A dosage of 25 mg/kg hexamethonium bromide (Sigma-Aldrich, St. Louis, MO) was injected into the femoral vein to induce a depressor effect. The maximal decrease in MAP was considered as the index of sympathetic nerve activity (SNA).

2.5. Western blot analysis

Specimens for Western blot were dissected fresh. Western blot analyses for matrix metalloproteinase-2 (MMP-2), MMP-9 and GAPDH concentrations were performed in a standard fashion. Briefly, aortic tissues were homogenized and cells were harvested with trypsin, and the protein extract were loaded on to a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were then transferred onto polyvinyl-difluoride membranes. Membranes were probed with primary antibody for MMP-2, MMP-9 and GAPDH as a loading control. Enhanced chemiluminescence (Servicebio, Wuhan, Hubei, China) was used to visualize the protein bands. The intensity of these bands was quantitated using AlphaEaseFC 4.0 software from Alpha Innotech Corporation.

2.6. Histologic examinations

The dissected aorta taken for histologic examinations to confirm the formation of aorta dissection was immersion-fixed in 10% neutral-buffered formalin. These specimens were cut into 4 μm-thick sections and were stained with hematoxylin and eosin (H & E), Elastica van Gieson (EVG).

2.7. Statistical analysis

Continuous variables were expressed as mean ± standard deviation. Statistical Product and Service Solutions 19.0 software (SPSS) was used for all analyses. Nonparametric data were analyzed using chi-square tests. One-way analysis of variance (ANOVA) was utilized to compare parametric variables among the groups. The level of significance was chosen to be $P < 0.05$.

3. Results

3.1. Effects of SCGx on the formation of BAPN-induced AD

Among the rats that only received the administration of BAPN, nine rats survived until the end of the study. Six rats dying prematurely owing to dissecting aorta rupture and extensive blood clots were discovered in their thoracic cavities (Fig. 1A). Of the surviving rats in BAPN group, four rats were found the formation of AD, all located from the ascending aorta to the proximal descending aorta (Fig. 1B, C). In BAPN + SCGx group two rats died of aortic rupture during the study and only one of the surviving rats developed AD. The incidence of AD was decreased by SCGx treatment from 66.7% (10/15) to 20% (3/15) ($P < 0.05$).

3.2. Effects of BAPN administration with or without SCGx on body weight

Mean body weights of BAPN and BAPN + SCGx groups during the course of the experiment were lower than the control group (Fig. 2A). BAPN treatment significantly suppressed the increase in the body weight when compared with control group, whereas SCGx treatment alleviated this effect (92.9 ± 30.1 g; 211.6 ± 8.2 g and 122.1 ± 25.9 g; Fig. 2B).

3.3. Effects of BAPN administration with or without SCGx on MAP, HR and SNA

Our results showed that there was no significant difference in MAP between the three groups (91.9 ± 11.2 vs 87.2 ± 6.4 vs 91.7 ± 11.4 mm Hg; Fig. 3A).When compared with BAPN group, rats in BAPN + SCGx group showed a significant decrease in HR (364.5 ± 18.6 vs 275.7 ± 51.6 bpm; Fig. 3B). The representative recording of effects of hexamethonium on MAP is shown in Fig. 4. Hexamethonium significantly lowered the MAP in BAPN treated rats compared with BAPN + SCGx treated rats and control rats (−43.9 ± 7.3 vs −24.1 ± 4.5, $P < 0.05$; −43.9 ± 7.3 vs −31.4 ± 6.7 mm Hg, $P < 0.05$; Fig. 4D), which demonstrates that there is significant elevation of SNA in BAPN treated rats while SCGx treatment significantly inhibited the effect.