

*Full Paper***Adenine Sulfate Improves Cardiac Function and the Cardiac Cholinergic System After Myocardial Infarction in Rats**

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Abstract. Recent studies have shown that vagal activation may have an important therapeutic implication for myocardial infarction (MI), but effective strategies remain unexplored. Here, we investigate whether adenine sulfate can preserve cardiac function and the cholinergic system against MI. Rats were treated with adenine sulfate for three weeks after coronary ligation. Cardiac function was assessed by hemodynamics. The muscarinic M₂ receptor and cholinesterase-positive nerves were semi-quantified by immunochemical and histochemical staining. The maximal binding capacity (B_{max}) of muscarinic receptors, determined by radioligand binding assay, showed that cardiac function was impaired in MI rats. Adenine sulfate reversed MI-induced reduction of mean artery pressure and left ventricular systolic pressure and elevation of left ventricular end-diastolic pressure. Moreover, adenine sulfate also increased nitric oxide (NO) and nitric oxide synthase (NOS) activity. The amelioration was accompanied by a reversal of the infarction-induced reduction of cholinesterase-positive nerves and M₂-receptor expression and B_{max} in the adenine sulfate high dose group. Meanwhile, adenine sulfate treatment corrected the disorder of cardiac redox state by reduction in maleic dialdehyde and increase in superoxide dismutase. In conclusion, adenine sulfate exerts cardioprotection against MI and ameliorates NO production. Changes in cardiac vagal distribution density and M₂-receptor expression raise the possibility that improvement of the cardiac cholinergic system is involved in adenine sulfate-induced cardioprotective effects.

Keywords: adenine sulfate, cardioprotection, muscarinic receptor, nitric oxide, myocardial infarction

Introduction

Cardiac function is regulated by a dynamic interaction with the sympathetic and parasympathetic (vagal) nervous systems (1). About three quarters of myocardial ischemic events are triggered by the imbalanced autonomic nervous system, appearing as suppressed cardiac vagal activity in combination with increased sympathetic activity (2). Clinical studies have shown that impaired vagal function is related to poor outcome and high mortality of patients with post-infarction heart failure. Accordingly, the extent of vagal withdrawal is proved to be a prognostic marker in myocardium infarction (MI).

Also, heart dysfunction exacerbated the disorder of cardiac neuromodulation: excess norepinephrine release (3), decreased heart rate variability (4), and nerve remodeling (5).

To solve the problem of autonomic nerve disorder after MI, two options of treatment have been put forward: inhibiting the overactive sympathetic nerves and/or stimulating the vagal nerves. Various therapeutic agents, including beta-blockers (6, 7), angiotensin-converting enzyme inhibitors (8), and angiotensin-receptor antagonists (9) have been proven to be useful pharmacotherapy mainly by correcting the abnormally augmented sympathetic activity. In contrast to the extensive studies on the sympathetic nerve in cardiac dysfunction, however, less attention has been devoted to actively remedying the reduced vagal activity. Recent investigations have found that vagal stimulation exerts an antiarrhythmic effect in

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the early phase of acute myocardial ischemia (10), modulates myocardial redox state (11), prevents cardiac remodeling, and improves the survival rate of chronic heart failure after MI (12). These suggest that enhancement of vagal tone contributes to improving cardiac function, and vagal activation may hold future promise as a therapeutic option and preventive action for MI (13), but an effective strategy to do so remains unexplored up to now. Because of its invasive nature, widespread use of vagal stimulation in clinical practice may be difficult. Therefore, the use of specific cholinergic drugs may provide a feasible means of effective cardioprotection clinically.

Adenine sulfate (the exogenous precursor of adenosine) can synthesize adenosine after entering myocardial cells (14), which successfully simulates adenosine preconditioning. However, the precise mechanism for this effect is still not clear. Thus, the objective of the present study is to investigate whether adenine sulfate plays a role in the adjustment of cardiac function and improves the cardiac cholinergic system against MI.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 180–220 g supplied by the Experimental Animal Center of Xi'an Jiaotong University were used in accordance with the Guidelines on the Care and Use of Laboratory Animals issued by the Chinese Council on Animal Research. The study was approved by the ethical committee of Xi'an Jiaotong University.

Experimental myocardial infarction

After induction of anesthesia with 3% sodium pentobarbital (40 mg/kg, intraperitoneal injection), rats were artificially ventilated and connected to an ECG recorder. Then left thoracotomy and left coronary ligation were performed. Successful ligation was confirmed by elevation of the S-T segment of lead II. In sham-operated group, rats underwent the same surgery without ligating the suture. Two weeks after surgery, rats were randomly allocated to groups treated with adenine sulfate solution (1.5, 2.0, or 2.5 mg/kg, once daily, subcutaneous injection) or saline.

Hemodynamic measurement

After three weeks of treatment, hemodynamic studies were performed with a polygraph recorder (Powerlab/4SP; AD Instruments, Sydney, NSW, Australia). After anesthesia, polyethylene catheter was inserted into the right common carotid artery and left ventricle. At the end of hemodynamic monitoring, a small region at the margin of the infarct was selected and directly fixed in 10%

neutral-buffered formaldehyde solution and the remainder of the heart was quickly removed and immediately frozen in liquid nitrogen then stored at -80°C for subsequent tests.

Infarct size measurement

The heart was frozen at -20°C for 30 min and then sliced into sequential 2-mm-thick sections from the apex to base. The slices were incubated in 1% 2,3,5-triphenyl tetrazolium chloride (TTC) solution for 15 min (37°C) and immersed in 4% formalin for 30 min to delineate the infarct areas more clearly. The surviving myocardium was stained brick red and the infarcted myocardium was pale white. Then slices were photographed and analyzed by Image Pro Plus 5.1 software (Media Cybernetics, Silver Spring, MD, USA). Percent infarct size was calculated as the ratio between the infarct area and the total area of the left ventricle.

Biochemical assays

Briefly, heart tissue was homogenized and centrifuged at $10,000 \times g$ at 4°C for 10 min. The protein content in the supernatant was detected by the Coomassie Brilliant Blue method, and then the resultant supernatant was aliquoted and stored at -80°C until analysis.

Nitric oxide (NO) production was determined indirectly as the concentration of nitrite from nitrates. Nitrate was converted to nitrite with aspergillus nitrite reductase and the total nitrite level was then measured with the Griess reagent (Nanjing Jiancheng Bioengineering Institute, NJBI, Nanjing, China). After incubation for 15 min at room temperature, the concentration of the resultant chromophore was determined at 550 nm using a spectrophotometer.

Nitric oxide synthase (NOS) activity was determined using a NOS-detection assay kit (NJBI) according to the manufacturer's instructions. This kit measures nitrite levels. The absorbance was determined at 530 nm. The results were expressed as nanomoles of NO released per milligram of protein (nmol/mg protein).

Maleic dialdehyde (MDA) and superoxide dismutase (SOD) activity were measured spectrophotometrically at 532 and 550 nm absorbance using their corresponding detection assay kits (NJBI), which indirectly reflected the oxidant and antioxidant levels of the heart.

Histological staining

Myocardia were fixed in 10% formalin and then cut with a cryostat (Leica, Bensheim, Germany) into 20- μm -thick tissue sections. Histochemical analysis of cholinesterase-positive nerves was performed using a modification of a method previously described (15). Frozen sections were fixed in cold 10% formalin containing 1%

CaCl₂ for 2 h and then incubated overnight at 4°C in a medium containing: 25 mg of acetylcholine iodide, 32.5 mL of 0.1 mol/L acetate buffer, 2.5 mL of 0.1 mol/L sodium citrate, 5 mL of 30 mmol/L CuSO₄, 1 mL of 4 mmol/L tetraisopropylpyrophosphoramidate, 5 mL of 5 mmol/L potassium ferricyanide, and 4 mL of distilled water. The distribution density of the cholinesterase-positive nerves was calculated using a modified point-counting method.

Immunohistochemistry staining

Frozen sections (6- μ m-thick) were post-fixed in cold acetone for 10 min and then treated with 3% H₂O₂ for 30 min to inhibit endogenous myocardial peroxidase activity. After blocking nonspecific protein binding sites, the sections were incubated with a primary antibody against rat M₂ (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) overnight at 4°C. The sections were then washed with phosphate buffer solution and incubated in biotin-conjugated rabbit anti-goat immunoglobulin at 37°C for 30 min. The slides were rinsed and reacted with a streptavidin-biotin complex conjugated with peroxidase at 37°C for 30 min. Visualization of the chromogen (horseradish peroxidase) was performed by incubating the sections with a 3, 3'-diaminobenzidine kit. Finally, the slides were dehydrated, cleared, mounted, and then examined with a high-resolution CCD camera and analyzed with Image Pro Plus 5.1 software (Media Cybernetics) for semi-quantitative determination of the percent area occupied by M₂ receptors.

Radioligand receptor binding assay

Myocardial tissues were cut into small pieces and added to ice-cold Tris-HCl buffer and then homogenized at 4°C. The crude homogenate was centrifuged at 800 \times g for 10 min to remove cellular debris. The supernatant was filtered through cheesecloth and then centrifuged at 20,000 \times g for 30 min. The resulting pellet was gently resuspended in the above buffer and samples were removed for protein determination using the Coomassie Brilliant Blue method. Membrane suspensions from either the atrium or ventricles (100 μ L) were incubated with increasing concentrations of [³H]QNB (0.1–6 nmol/L) to measure total binding. Non-specific binding was defined in the presence of 2 μ mol/L atropine. Specific binding was estimated as the difference between total and non-specific binding. Binding reactions were performed for 2 h and terminated by addition of 3 mL ice-cold 50 mmol/L sodium phosphate buffer. Membranes were filtered using a tissue harvester through GF/B filters. Individual filters were washed three times with ice-cold buffer and dried in an incubator at 80°C. Once dried, 5 mL of scintillation liquid (23 mmol/L PPO,

0.8 mmol/L POPOP, 10% v/v Triton X-100 dissolved in toluene) was added. Filters were kept in a dark dry room for 12 h, after which radioactivity was measured by liquid scintillation spectrometry.

Statistical analyses

All the data were presented as mean \pm S.E.M. Differences were evaluated by one-way factorial analysis of variance (ANOVA) followed by a post hoc Tukey test. Significance level was set at $P < 0.05$.

Results

Adenine sulfate improved cardiac hemodynamics and reduced infarct size

After three weeks of treatment, hemodynamics was measured in vivo in anesthetized rats. As expected, cardiac function was significantly impaired in MI rats (Fig. 1: A–C). The mean artery pressure (MAP) and left ventricular systolic pressure (LVSP) were both markedly diminished and left ventricular end-diastolic pressure (LVEDP) was elevated in MI rats compared with those in sham-operated rats (all $P < 0.01$). Importantly, MI rats treated with adenine sulfate at 2.0 or 2.5 mg/kg showed 23%, or 37% increase in MAP and 31%, or 39% increase in LVSP, respectively, compared with untreated MI rats. Adenine sulfate treatment also led to a reduction in LVEDP with respect to MI ($P < 0.05–0.01$). As indexes of myocardial contractility and relaxation, the maximal rates of increase and decrease in left ventricular pressure ($+dP/dt_{\max}$ and $-dP/dt_{\max}$) were both impaired in MI rats compared to those in the sham-operated group ($P < 0.01$ for both, Fig. 1: D and E). MI rats treated with adenine sulfate (2.0 or 2.5 mg/kg) showed an improved contractility and relaxation compared to MI rats ($P < 0.01$). However, in the adenine sulfate low dose (1.5 mg/kg) group, the differences of hemodynamic parameters hardly reached statistical significance compared to those in the untreated MI group. To further evaluate the effect of adenine sulfate on MI, infarct size was measured by TTC staining. The infarct size was significantly reduced in rats subjected to adenine sulfate (2.5 mg/kg) treatment compared to that in the sham group ($25.0 \pm 3.5\%$ vs. $42.8 \pm 4.7\%$; $P < 0.01$, Fig. 1F).

Adenine sulfate increased NO concentration and NOS activity

NO concentration and NOS activity in myocardial homogenates were markedly decreased in the MI group compared to the sham-operated rats (both $P < 0.01$). Most notably, treatment with adenine sulfate for three weeks resulted in a reversal of the infarction-induced reduction in both NO concentration (Fig. 2A) and NOS

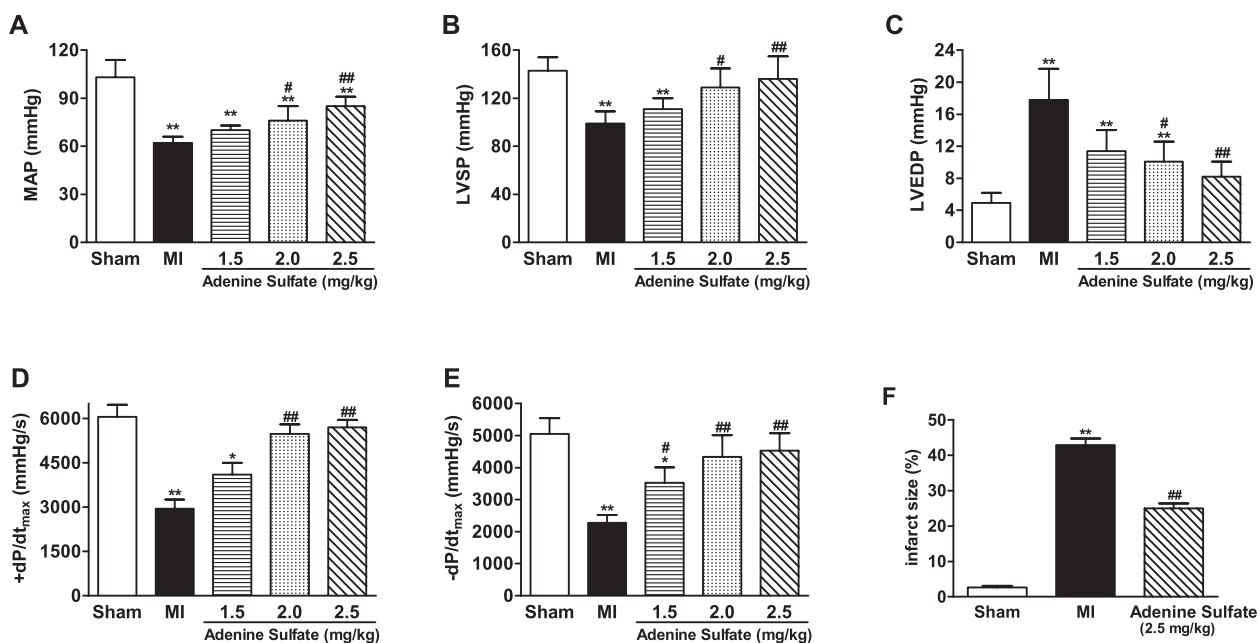


Fig. 1. Effects of adenine sulfate on cardiac function at three weeks after infarction. A) MAP, mean artery pressure; B) LVSP, left ventricular systolic pressure; C) LVEDP, left ventricular end-diastolic pressure; D) $+dP/dt_{\max}$: the maximal rates of increase in left ventricular pressure; E) $-dP/dt_{\max}$: the maximal rates of decrease in left ventricular pressure; F) infarct size. MI, myocardium infarction. Values are expressed as the mean \pm S.E.M. ($n = 8$). All treatment groups were assessed simultaneously using one-way ANOVA for individual parameters. * $P < 0.05$, ** $P < 0.01$ vs. Sham group; # $P < 0.05$, ## $P < 0.01$ vs. MI group.

activity (Fig. 2B). NO level was elevated from 10.43 ± 2.5 (MI) to 22.75 ± 2.5 , 23.36 ± 2.7 , and 26.24 ± 2.3 nmol/mg protein in the adenine sulfate-treated groups (1.5, 2.0, and 2.5 mg/kg, respectively) ($P < 0.01$ vs. MI). The NOS activity was increased by 47%, 59%, and 65% compared to the MI group (1.5 mg/kg, $P < 0.05$; 2.0, 2.5 mg/kg, $P < 0.01$).

Adenine sulfate increased distribution density of cholinesterase-positive nerves

Histochemical acetylcholine esterase (AChE) staining revealed that cholinesterase-positive nerves were distributed widely in all regions of the rat heart (Fig. 3). The counts of cholinesterase-positive nerves in the MI group were markedly lower than those in the sham-operated group in both the atria and ventricles ($P < 0.01$). Moreover, the number of counts in the adenine sulfate-treated groups (2.5 mg/kg) recovered compared to that in the MI group ($P < 0.05$, Fig. 4). Adenine sulfate therefore reversed the effects of MI on cholinergic nerves.

Adenine sulfate increased the expression of M_2 receptors

As shown in Fig. 5, A–F, the immunoreaction of M_2 receptors (colored yellow-brown) was observed around the periphery of the myocardial cells in the cross-sections.

M_2 receptors were observed in all regions of the rat heart. The expression of M_2 receptors appeared to be weaker in the MI rat heart than in the sham-operated group ($P < 0.01$, Fig. 6). Only the adenine sulfate high dose group (2.5 mg/kg) showed enhanced expression of M_2 receptors in both the atria and ventricles; compared to the expression in the MI groups, the increases in the treated group were pronounced (in atria, $P < 0.01$ and in ventricles, $P < 0.05$; Fig. 6). Low doses of adenine sulphate (1.5, 2.0 mg/kg) had no discernable effect on M_2 -receptor expression compared with the MI group. Moreover, in the lowest dose of adenine sulfate (1.5 mg/kg) group, M_2 -receptor expression in atria was significantly lower than that in sham-operated rats ($P < 0.05$). Thus, high dose of adenine sulfate enhanced the expression of M_2 receptors, which was down-regulated after MI.

Adenine sulfate increased the binding capacity of muscarinic receptors

A radioligand receptor binding assay was used to evaluate muscarinic receptor binding ability. Scatchard analysis of the saturation curves estimated the density of muscarinic receptors in terms of the maximal binding capacity (B_{\max}). B_{\max} of muscarinic receptors was decreased dramatically compared with that in the sham-operated group ($P < 0.01$). Adenine sulfate (2.5 mg/kg)

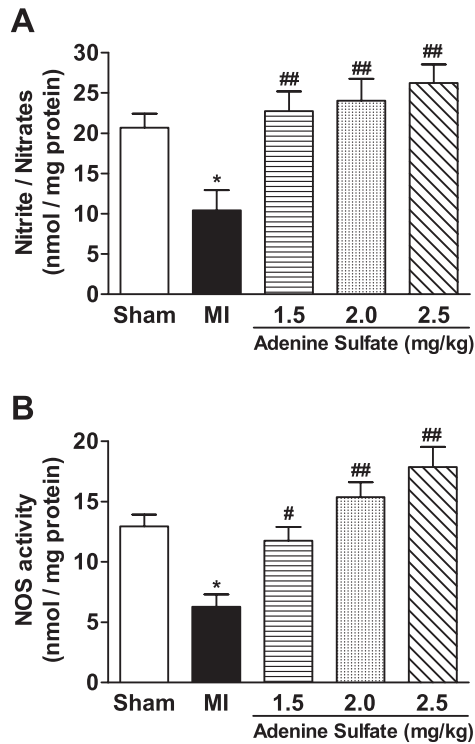


Fig. 2. Effects of adenine sulfate treatment on NO (determined as nitrite/nitrate) (A) and NOS activity (B) in myocardial homogenates of rats. Adenine sulfate increased the infarct-induced reduction of NO concentration and NOS activities. MI, myocardium infarction. Data are reported as the mean \pm S.E.M., $n = 8$. * $P < 0.05$ vs. Sham group; # $P < 0.05$, ## $P < 0.01$ vs. MI group.

significantly increased B_{\max} compared with that in the MI group (249 ± 9 vs. 169 ± 11 fmol/mg in atria and 95 ± 6 vs. 53 ± 3 fmol/mg in ventricles, $P < 0.01$; Fig. 7). Meanwhile, adenine sulfate (2.0 mg/kg) just increased B_{\max} in atria with respect to that in the MI group ($P < 0.01$, Fig. 7). Thus, adenine sulfate enhanced the function of muscarinic receptors, which was impaired after MI. Moreover, the effect of adenine sulfate on atria was more sensitive than in ventricles.

Adenine sulfate reduced MDA and increased SOD activity

The redox state of the myocardium was evaluated by measuring the levels of MDA and SOD. As expected, compared with the sham-operated group, the MI group had dramatically higher level of MDA and lower level of SOD ($P < 0.01$). However, MDA was reduced (Fig. 8A) and SOD was increased (Fig. 8B) after the treatment with adenine sulfate. Especially in the adenine sulfate high dose (2.5 mg/kg) group, MDA dropped to 2.77 ± 0.15 compared with 4.25 ± 0.26 nmol/mg protein in the MI group ($P < 0.01$ vs. MI) and SOD reached to 170 ± 8 compared with 111 ± 5 U/mg protein in the MI group ($P < 0.01$ vs. MI).

Discussion

In the present study, we have shown that after MI, cardiac function was severely impaired and NO levels and NOS activity were significantly decreased, which

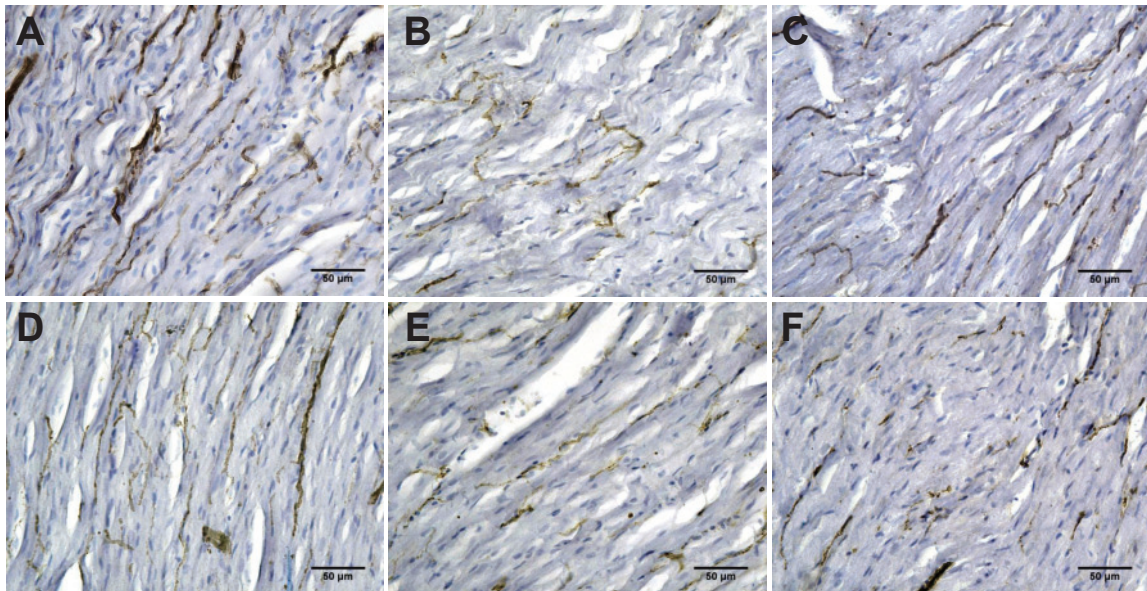


Fig. 3. Photomicrographs of staining for cholinesterase-positive nerves of rat atria (A – C) and ventricles (D – F) from the sham group (A, D), myocardial infarction group (B, E), and 2.5 mg/kg adenine sulfate-treated groups (C, F). Scale bar, 50 μ m.

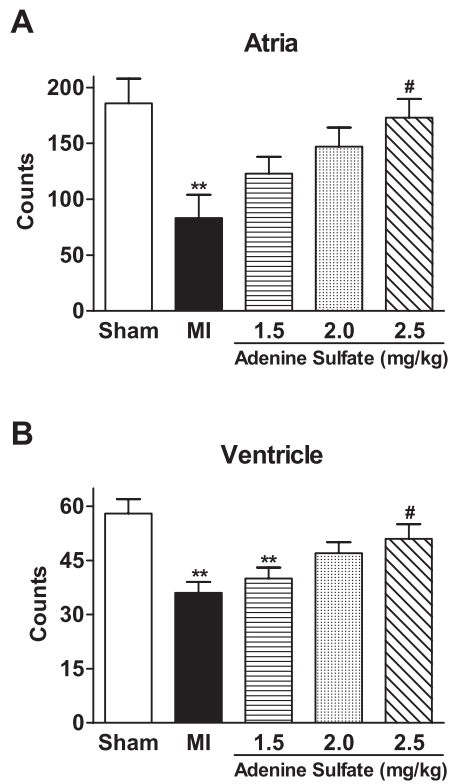


Fig. 4. The distribution density of cholinesterase-positive nerves in atria and ventricles. Adenine sulfate (2.5 mg/kg) recovered the number of counts of cholinesterase-positive nerves. MI, myocardium infarction. Data are reported as the mean \pm S.E.M., $n = 8$. ** $P < 0.01$ vs. Sham group and # $P < 0.05$ vs. MI group.

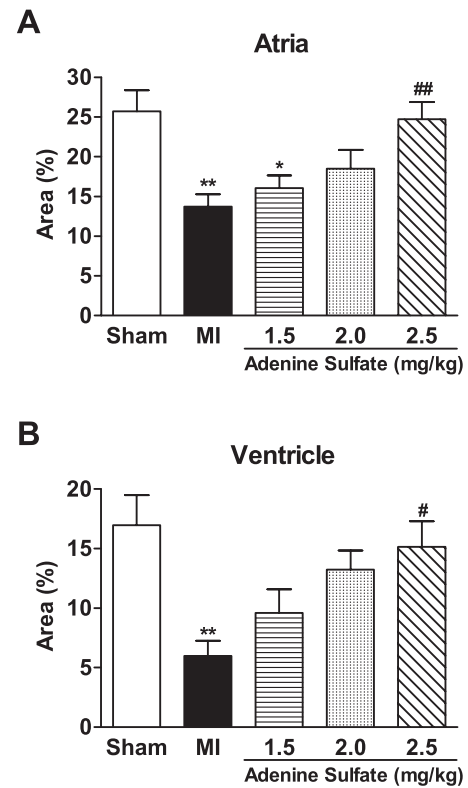


Fig. 6. The percent of area that is immunopositive for M₂ receptors in atria and ventricles. Adenine sulfate (2.5 mg/kg) enhanced the expression of muscarinic M₂ receptors both in atria and in ventricles. MI, myocardium infarction. Data are reported as the mean \pm S.E.M., $n = 8$. * $P < 0.05$, ** $P < 0.01$ vs. Sham group; # $P < 0.05$, ## $P < 0.01$ vs. MI group.

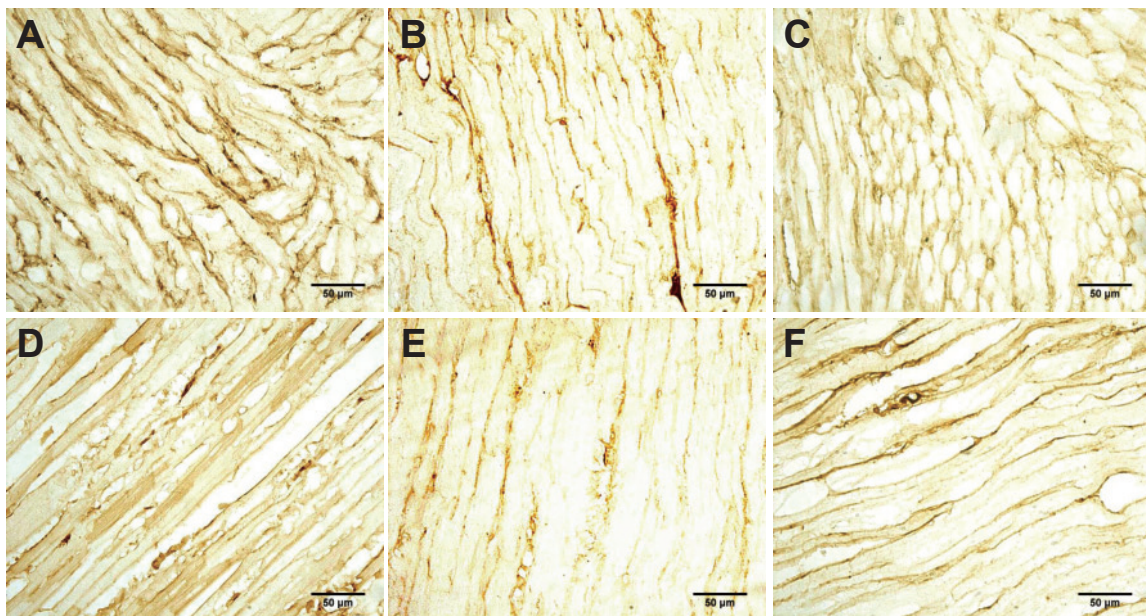


Fig. 5. Photomicrographs of streptavidin-biotin complex immunocytochemical staining for muscarinic M₂ receptors in rat atria (A – C) and ventricles (D – F) from the sham group (A, D), myocardial infarction group (B, E), and 2.5 mg/kg adenine sulfate-treated groups (C, F). Scale bar, 50 μ m.

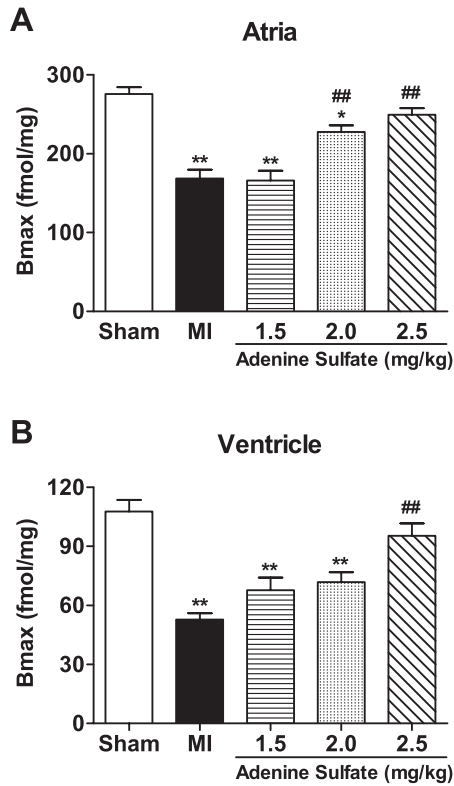


Fig. 7. The density of muscarinic receptors in atrial and ventricular myocardium of rats. Adenine sulfate (2.5 mg/kg) significantly increased B_{max} of muscarinic receptors. MI, myocardium infarction. Data are reported as the mean \pm S.E.M., $n = 8$. * $P < 0.05$, ** $P < 0.01$ vs. Sham group; ## $P < 0.01$ vs. MI group.

was accompanied by the down-regulation of the cholinergic nerve system and alteration of cardiac redox homeostasis. More importantly, after treatment with adenine sulfate for three weeks, cardiac function was pronouncedly improved and NO production was ameliorated as well. Furthermore, adenine sulfate (2.5 mg/kg) obviously reversed the infarction-induced harmful influence on cholinergic nerves and expression of M_2 receptors and corrected the disorder of cardiac redox state. It could be postulated that improvement of the cholinergic system in post-infarction myocardium may be the indirect effects of NO amelioration induced by adenine sulfate treatment.

The cardiac autonomic nervous system plays a critical role in heart rate and cardiac output, but myocardial infarction disrupts the normal neural regulation of the heart, thus resulting in an almost complete withdrawal of tonic vagal activity with increased sympathetic activity (2, 16, 17). Keeping the balance appears to be important for maintaining normal hemodynamic homeostasis. Vagus nerve functions through neurotransmitter acetylcholine releasing from nerve endings and then interacting

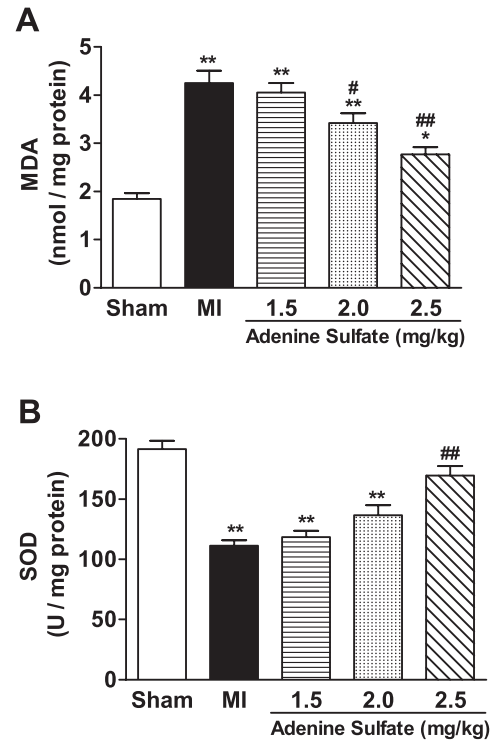


Fig. 8. Effects of adenine sulfate treatment on MDA (A) and SOD activity (B) in myocardial homogenates of rats. Adenine sulfate (2.5 mg/kg) reduced MDA concentration and increased SOD activity. MI, myocardium infarction. Data are reported as the mean \pm S.E.M., $n = 8$. * $P < 0.05$, ** $P < 0.01$ vs. Sham group; # $P < 0.05$, ## $P < 0.01$ vs. MI group.

with muscarinic acetylcholine receptor. Thus the number of cholinergic nerves and the expression of receptors may indirectly reflect the vagus nerve function. In our experiment, immunohistochemical results showed that the expression of muscarinic receptors and cholinergic nerves were down-regulated both in the atria and ventricle in rat hearts subjected to MI (Figs. 3, 5). Furthermore, the maximal binding capacity of the muscarinic receptor was impaired in infarcted myocardium (Fig. 7). These changes are in line with the previous studies, which demonstrated both muscarinic receptor density and functional responsiveness were decreased in rat model of MI or heart failure induced by ethanol (18), adriamycin (19), or aortic banding (20).

Adenosine is perhaps the most widely studied "cardioprotectant" that limits cellular death and dysfunction following ischemia insult (21). However, the defect of ultrashort half-life greatly restricts its clinical availability. Several lines of evidence have shown that adenosine provides cardioprotection by attenuating catecholamine release (22). Nonetheless, there was no available experimental research on the effects of adenosine (or its ana-

logue) on the cardiac cholinergic system. Adenine sulfate (the exogenous precursor of adenosine) can synthesize adenosine after entering myocardial cells (14), which successfully simulates adenosine preconditioning. Besides, adenine sulfate is a simpler chemical product and much more soluble compared with adenosine. Moreover, salts of adenine have been given to rabbits and found to be non-toxic when administered either intravenously or intramuscularly. Although they are known to have effects on the cardiovascular system and the peripheral circulation, the physiological and therapeutic properties of adenine and its derivatives have not yet been completely elucidated. Our results demonstrate that adenine sulfate reversed the ischemia-induced down-regulation of cholinergic nerves (Fig. 4) and improved muscarinic receptor expression and binding capacity in the post-infarction myocardium (Figs. 6 and 7). This means that adenine sulfate not only enhances the muscarinic receptors expression, but also improves the muscarinic receptor function. The amelioration of cardiac function with the improvement of the cholinergic system might be a potential new mechanism of for the action of adenine sulfate, which also provides a novel way to increase the vagal tone after MI. Therefore, adenine sulfate (as a cholinomimetic) may become a promising candidate for the treatment of ischemic heart diseases.

Emerging evidence suggests that many neuromodulators that are intrinsic to intracardiac ganglia can influence cardiac autonomic function (23). These act as cotransmitters between neuronal populations or are released from the myocardium itself (24). Extensive studies have demonstrated that NO as the neuromodulator of parasympathetic nerves has a distinct effect on facilitating the actions of vagal nerves by augmenting the release of acetylcholine and reducing the downstream action of catecholamine on heart rate and contractility (25, 26). In our experiment, adenine sulfate (1.5, 2.0, 2.5 mg/kg) significantly increased NO concentration and NOS activity, which showed a tendency of dose-relative cardioprotection, although the difference of these changes did not reach statistical significance among groups (Fig. 2). However, only the high dose of adenine sulfate (2.5 mg/kg) improved the cholinergic system markedly. It is speculated that besides the direct cardiac protective effects, NO (maybe need to reach a certain concentration) is responsible for adenine sulfate-induced protective effects indirectly via amelioration of the cholinergic system.

Adenine is converted to adenosine and the effect of adenine mimics that of adenosine, which implies that the effects of adenine are mediated by adenosine and its corresponding receptors. Recently, it has been proven that adenosine is also able to directly produce NO in

cardiomyocytes to prevent the mitochondrial oxidant damage through activation of NOS (27). The adenosine A₂-receptor antagonist 8-(3-chlorostyryl) caffeine prevented the increase in NO production but not the A₁ antagonist. In addition, CGS21680, an adenosine A₂-receptor agonist, markedly increased NO, further supporting the involvement of A₂ receptors. Our results showed that adenine sulfate also increased the NO production and NOS activity. Based on these studies and our experiment, we speculated that adenosine A₂ receptor might play a role in adenine sulfate-induced cardioprotection. Nevertheless, the mechanism underlying the link between adenine sulfate and adenosine receptor still needs further investigation.

Recently it was reported that electrical stimulation of cardiac parasympathetic nerve suppressed the enhanced reactive oxygen species generation in the failing heart and then altered the myocardial redox status via muscarinic receptors in mice after MI (11). Moreover acetylcholine, the principle vagal neurotransmitter, had a direct action against free radical generation in the myocardium. In our experiment, adenine sulfate reduced MDA as an index of reactive oxygen species (28) and increased SOD activity in ischemic myocardium. These alterations were in parallel with the amelioration of the cholinergic system as a result of treatment with adenine sulfate (2.5 mg/kg). Based on our results and others, it is speculated that adenine sulfate-induced cardioprotection is associated with reduced oxidant stress through the promotion of the expression and function of the cardiac cholinergic system.

In summary, the present study has suggested that adenine sulfate reduced cardiac dysfunctions related to coronary ligation. These benefits were associated with improvement of NO production followed by preservation of the cardiac cholinergic system and reduction in oxidative stress after MI.

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