# Effect of lysophosphatidic acid on the immune inflammatory response and the connexin 43 protein in myocardial infarction

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**Abstract.** Lysophosphatidic acid (LPA) is an intermediate product of membrane phospholipid metabolism. Recently, LPA has gained attention for its involvement in the pathological processes of certain cardiovascular diseases. The aim of the present study was to clarify the association between the effect of LPA and the immune inflammatory response, and to investigate the effects of LPA on the protein expression levels of connexin 43 during myocardial infarction. Surface electrocardiograms of myocardial infarction rats and isolated rat heart tissue samples were obtained in order to determine the effect of LPA on the incidence of arrhythmia in rats that exhibited changes in immune status. The results demonstrated that the incidence of arrhythmia decreased when the rat immune systems were suppressed, and the incidence of arrhythmia increased when the rat immune systems were enhanced. The concentration levels of tumor necrosis factor (TNF)-α were determined by ELISA, and the results demonstrated that LPA induced T lymphocyte synthesis and TNF-α release. Using a patch-clamp technique, LPA was shown to increase the current amplitude of the voltage-dependent potassium channels ($K_v$) and calcium-activated potassium channels ($K_{Ca}$) in Jurkat T cells. The protein expression of connexin 43 (Cx43) was determined by immunohistochemical staining. The results indicated that LPA caused the degradation of Cx43 and decreased the expression of Cx43. This effect was associated with the immune status of the rats. There was a further decrease in Cx43 expression in the rats of the immune-enhanced group. To the best of our knowledge, these results provide the first evidence that LPA causes arrhythmia through the regulation of immune inflammatory cells and the decrease of Cx43 protein expression. The present study provided an experimental basis for the treatment of arrhythmia and may guide clinical care.

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*Key words:* lysophosphatidic acid, connexin 43 protein, immune inflammatory response, acute myocardial infarction, arrhythmia

## Introduction

Acute myocardial infarction (AMI) is a disease that severely affects the health and life quality of patients. Arrhythmia is a complication of myocardial infarction, which is one of the most severe cardiovascular diseases, and it is the predominant cause of myocardial infarction-associated mortality (1). Since patients with ischemic heart disease are particularly prone to arrhythmias, they are often admitted for arrhythmia monitoring (2). Lysophosphatidic acid (LPA), which is an intermediate product of membrane phospholipid metabolism, is a lipid mediator with various biological functions that are predominantly mediated by specific G protein-coupled receptors (3). As a water-soluble glycerol phospholipid with a simple structure, LPA is secreted from numerous cell types, including platelets, fibroblasts and ovarian cancer cells (4). The concentration of LPA in regional myocardial tissue and plasma increases during myocardial infarction, and it can also cause arrhythmia (5).

During myocardial infarction, both the infarcted area and the non-infarcted area exhibit perivasculitis, and the infiltration of a large number of inflammatory cells can be observed (6,7). A variety of inflammatory factors, including interferon-γ, interleukin (IL)-4, IL-5, IL-6, IL-8 and IL-10, increase in the peripheral blood, indicating that myocardial infarction is a non-infectious immune inflammatory process (8,9). The immune inflammatory response has an important role in myocardial infarction (10,11). Numerous studies have focused on the effect of LPA on myocardial ischemia, but there are no reports on the immune regulatory effect of LPA on connexin 43 (Cx43) protein expression and arrhythmias (1,4,12). In the present study, various experimental methods were used to observe the effects of LPA on tumor necrosis factor (TNF)-α, Cx43 and potassium channels. The mechanism underlying the LPA-induced induction of arrhythmia was also investigated.

## Materials and methods

*Experimental animals*. A total of 80 healthy adult male and female Wistar rats (clean grade), aged 8-10 week and weighing 240-260 g, were purchased from the Experimental Animal Center of Jilin University (Changchun, China). Rats were maintained in an animal care facility at 21-23˚C, relative humidity (55±5%) with a 12-h light/dark cycle and *ad libitum* access to food and chow for at least three days prior to the initiation of the experiment. The procedures used in the present study were approved by the Animal Ethics Committee of The First Hospital of Jilin University, in accordance with the Guide for the Care and Use of Laboratory Animals issued by the US National Institutes of Health (13). All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

*Surface electrocardiogram (ECG)*. A total of 40 rats were randomly and equally divided into 4 groups: A sham-operated group, an AMI model group, an immune-enhanced group (treated with 1.25 mg/kg thymopeptide (H20003884; Livzon Pharmaceutical Group, Inc., Shanxi, China) by intraperitoneal injection) and an immune-suppressed group (treated with 15 mg/kg cyclophosphamide (H32020857; Jiangsu Hengrui Medicine Co., Ltd., Lianyungang, China) by intraperitoneal injection). For the 8 days leading up to the experimental procedures, the rats were maintained and fed, as outlined. The number of leukocytes was detected in the peripheral blood. Briefly, the rat tail was soaked at 45-50˚C for ~3 min to dilate the blood vessels and a 5 mm incision was made to harvest 1 ml blood. Full blood count analysis was performed within 2 h of collection using a Sysmex XE 2100 hematology analyzer (Sysmex UK, Milton Keynes, UK). If the number of leukocytes in the immune-enhanced group was $\geq 1.2 \times 10^9$/l and the number of leukocytes in the immune-suppressed group was $\leq 0.6 \times 10^9$/l, then immune intervention was considered successful.

Rats were anesthetized with 20 g urethane dissolved in 100 ml saline solution (0.9%) (1 g/kg; 5 $\mu$l/g body weight; U2500) by intraperitoneal injection, and a thoracotomy was performed in the left 4-5 intercostal space. A total of 10 $\mu$l LPA (0.5 g/l dissolved in 0.9% NaCl saline; L7260) was intravenously injected into the hearts of the AMI group, the immune-enhanced group and the immune-suppressed group for <5 sec. The thoracotomy was completed in 30 sec. The left anterior descending artery of coronar was ligated. The heart was placed back in the chest prior to being closed with sutures. Rats in the 4 groups were analyzed by surface lead II ECG (DECG-03A; Mindray Medical International, Ltd., Nanshan, China). Successful construction of the acute myocardial infarction model was observed as ST-segment elevation and the formation of a flag-shaped waveform of the T wave. ECG recordings were obtained and analyzed using a BL-420S Biological Function Recording system (Taimeng Technology Co., Ltd., Chengdu, China).

*Isolated rat heart perfusion*. A total of 40 rats were randomly and equally divided into 4 groups: A sham-operated group, an LPA group, an immune-enhanced + LPA group and an immune-suppressed + LPA group. Hearts were isolated and rapidly perfused with improved Krebs-Henseleit (K-H) solution in a Langendorff Perfusion system (Radnoti LLC, Monrovia, CA, USA). The perfusion pressure was 10.1 kPa, and the perfusion temperature was 37˚C. The improved K-H solution was saturated with 95% $O_2$ and 5% $CO_2$, and the composition of the solution was as follows: 118.0 mmol/l NaCl, 4.7 mmol/l KC1, 1.2 mmol/l $KH_2PO_4$, 1.2 mmol/l $MgSO_4$, 25.0 mmol/l $NaHCO_3$, 1.3 mmol/l $CaCl_2$, and 1.1 mmol/l glucose, with pH titrated to 7.2-7.4. After 10 min the isolated heart was functioning in a stable manner. LPA (5 $\mu$mol/l) was added to the K-H solution in the hearts of the LPA model group, the immune-enhanced + LPA group and the immune-suppressed + LPA group. Surface electrocardiograms were analyzed by surface lead II ECG (the needle electrode was inserted subcutaneously into the pulmonary cone and the cardiac apex). ECG recordings were obtained and analyzed using the BL-420S Biological Function Recording system. Subsequently, the number of premature ventricular contractions were observed.

*ELISA*. Jurkat T cells were purchased from the National Platform of Experimental Cell Resources (Sci-Tech, Beijing, China) and plated in a 96-well plate ($2 \times 10^6$ cells/ml) in 200 $\mu$l RPMI-1640 medium (R8755) supplemented with 10% fetal bovine serum (F2442) and 100 mg/ml penicillin/streptomycin (V900929), and incubated at 37˚C in atmosphere containing 5% $CO_2$ and 95% humidity. Jurkat T cells were divided into 3 groups: A control group, an LPA group (5 $\mu$mol/l), and a Ki16425 + LPA group (10 $\mu$mol/l and 5 $\mu$mol/l, respectively). Cell suspensions were obtained from each group following cell incubation for 1, 2, 4, 8, 12 and 24 h. The cell suspensions were centrifuged at 560 x g for 10 min at 22-23˚C, and the supernatants were collected. The concentration of TNF-α was determined using an ELISA kit (ERT2010-1; Assaypro LLC, St. Charles, MO, USA).

*Ionic current recordings*. Jurkat T cells ($2 \times 10^4$ cells/ml) were placed in a bath solution. Patch electrode pipettes were fabricated using a vertical pipette puller (Narishige PP-83; Narishige Scientific Instrument Lab., Tokyo, Japan). Pipettes with tip diameters of 1-2 $\mu$m had resistances of 3-6 MΩ when filled with the pipette solution. The pipette was placed on the cell surface using a microelectrode propeller (Narishige Scientific Instrument Lab.), and a high impedance seal (GΩ) was formed by vacuum suction. Subsequently, the negative pressure was increased in order to cause the clamp of the electrode tip to rupture, and the whole-cell patch clamp mode was formed. All experiments were performed at 20-22˚C. The voltage-clamp circuit was provided by a patch/whole-cell clamp amplifier (Dagan Total Clamp 8800; Dagan Corporation, Minneapolis, MN, USA). Pulse protocols and data acquisition were performed using a digital interface (TL-1 DMA interface; Axon Instruments, Foster City, CA, USA) coupled to an IBM compatible computer with PCLAMP 6.0 software (Molecular Devices LLC).

Ionic currents of $K_v$ were recorded under various voltage-clamp protocols of the step pulse: Holding potential -80 mV, test potentials -50 to +50 mV, step 10 mv, duration 300 ms, and frequency 1 Hz. The bath solution of $K_v$ contained the following components: 140 mmol/l NaCl, 4 mmol/l KCl, 1 mmol/l $MgC1_2$, 1 mmol/l $CaC1_2$, 10 mmol/l glucose and

Table I. Effects of lysophosphatidic acid on the incidence of arrhythmia in myocardial infarction rats.

| | | Prior to ligation | Following ligation | | | |
|---|---|---|---|---|---|---|
| Group | n | HR (beats/min) | Incidence of VPBs (%) | Initial VPB time (min) | HR (beats/min) | VPBs per hour |
| A | 0 | 322±21 | 0 | 0 | 328±15[a] | 0[a] |
| B | 7 | 324±15 | 70 | 30.5±6.3 | 422±46 | 10.1±2.4 |
| C | 8 | 321±17 | 80 | 24.2±5.8[b] | 463±55[b] | 13.5±3.9[b] |
| D | 5 | 324±31 | 50[a] | 41.2±8.9[b] | 384±57[b] | 7.2±2.9[b] |

[a]P<0.01 and [b]P<0.05 vs. group B (n=10; mean ± standard error of the mean). A, sham-operated group; B, AMI model group; C, immune-enhanced group; D, immune-suppressed group. HR, heart rate; VPB, ventricular premature beat.

Table II. Effects of LPA on the incidence of arrhythmia in isolated rat hearts.

| Group | n | Incidence of VPBs (%) | VPBs per 5 min |
|---|---|---|---|
| A | 10 | 0 | 0 |
| B | 10 | 80 | 5.2±3.9[a] |
| C | 10 | 90 | 7.4±3.7[a] |
| D | 10 | 50 | 3.5±3.8[b] |

[a]P<0.05, vs. group D and [b]P<0.01, vs. group A. A, sham-operated group; B, LPA model group; C, immune-enhanced + LPA group; D, immune-suppressed + LPA group. LPA, lysophosphatidic acid; VPB, ventricular premature beat.

10 mmol/l HEPES, pH 7.2 (filtered). The pipette solution of $K_v$ contained the following components: 90 mmol/l KCl, 50 mmol/l KF, 4 mmol/l NaCl, 4 $MgCl_2$, 0.5 mmol/l ethylene glycol tetraacetic acid (EGTA) and 10 mmol/l HEPES, pH 7.2 (filtered).

The bath solution of $K_{Ca}$ contained the following components: 160 mmol/l NaCl, 4.5 mmol/l KC1, 2 mmol/l $CaC1_2$, 1 mmol/l $MgC1_2$, 5 mmol/l HEPES, pH 7.4 (filtered). The pipette solution of $K_{Ca}$ contained the following components: 150 mmol/l K-asparatic acid, 2 mmol/l $MgCl_2$, 5 mmol/l HEPES, 10 mmol/l EGTA, 8.7 mmol/l $CaCl_2$, with a 1 $\mu$mol/l $Ca^{2+}$ concentration and pH 7.2 (filtered).

*Immunohistochemical staining*. Following the isolation of the hearts of the rats in the AMI, immune-enhanced and immune-suppressed groups were isolated (atria and blood vessels were discarded). The ventricles were fixed in 4% paraformaldehyde solution (252549) for 16-18 h. Fixed myocardial tissue samples were subsequently dehydrated in a graded ethanol series, cleared with xylene and embedded with paraffin (10152636; Guangjing Weiye Import & Export Co., Ltd., Tianjin, China). Tissue samples were cut into 3-$\mu$m sections using a sliding microtome (VT-1000S; Leica Microsystems GmbH, Wetzlar, Germany). One section was used for hematoxylin (H9627) and eosin (E4009) staining in order to observe the morphology of the myocardium, and the remaining two sections were used for immunohistochemical SP staining (SP-9000; Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China). Cx43 was observed in the tissue sections using an Olympus CKX41-A32PH inverted microscope (Olympus Corporation, Tokyo, Japan).

*Statistical analysis*. All data were analyzed using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) and are presented as the means ± standard error of the mean (n=4). The results obtained were compared using a t-test. Plots were generated using GraphPad Prism 6.01 (GraphPad Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

## Results

*Effects of LPA on the incidence of arrhythmia*. To determine the effects of LPA on the incidence of arrhythmia in rats that had altered immune status, ECGs of myocardial infarction rats and isolated rat hearts were obtained; the results demonstrated the presence of arrhythmias and ventricular premature beats (VPBs). Endogenous LPA enhanced the incidence of VPBs in rats of the immune-enhanced group and reduced the incidence of VPBs in rats of the immune-suppressed group (Table I).

ECGs of isolated rat hearts demonstrated that, following the addition of 5 $\mu$mol/l LPA to the K-H solution of the LPA model, immune-enhanced + LPA and immune-suppressed + LPA groups, the incidence of VPBs in the immune-enhanced + LPA group (90%) increased compared with that of the LPA model group (80%). The occurence of VPBs (7.4±3.7 times/5 min) also increased in the immune-enhanced + LPA group, as compared with the LPA model group (5.1±3.9 times/5 min). The incidence of VPBs in the immune-suppressed + LPA group (50%) significantly decreased compared with the LPA model group (80%; P<0.05), and occurrences (3.5±3.8 times/5 min) significantly increased compared with the LPA model group (5.2±3.9 times/5 min; P<0.05; Table II).

*Effects of LPA on TNF-α secretion*. The levels of TNF-α secreted by T lymphocytes significantly increased in the LPA group compared with the control group (P<0.01). Ki16425, which is a specific inhibitor of LPA, was able suppress the secretion of TNF-α (Table III). The concentration of TNF-α

Table III. Effects of LPA on tumor necrosis factor-α secretion in Jurkat T lymphocytes ($\mu$g/l).

| Group | 1 h | 2 h | 4 h | 8 h | 12 h | 24 h |
|---|---|---|---|---|---|---|
| Control | 0.77±0.04[a] | 0.76±0.05[a] | 0.81±0.04[a] | 0.77±0.08[a] | 0.78±0.04[a] | 0.79±0.03[a] |
| LPA | 3.22±0.18 | 3.96±0.19 | 5.20±0.29 | 4.73±0.27 | 4.19±0.30 | 4.32±0.18 |
| Ki16425 + LPA | 1.15±0.18[a] | 1.34±0.18[a] | 1.67±0.17[a] | 1.55±0.22[a] | 1.38±0.19[a] | 1.37±0.08[a] |

[a]P<0.01, vs. the LPA group, (means ± standard error of the mean; n=4). LPA, lysophosphatidic acid.

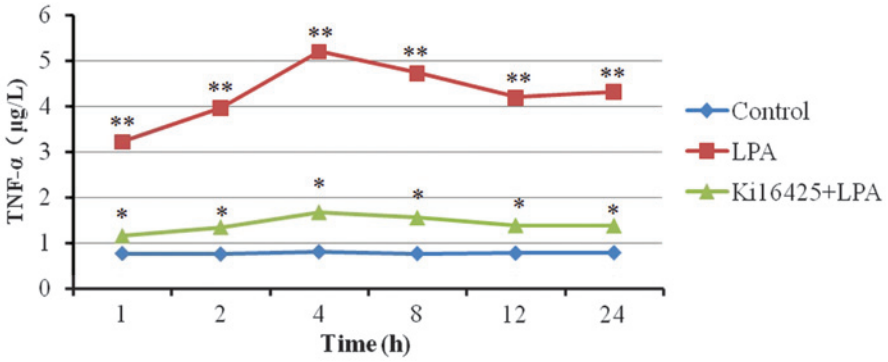


Figure 1. TNF-α concentrations markedly increase shortly after the addition of LPA to the Jurkat T lymphocytes. TNF-α concentrations peaked at 4 h, and stabilized within 24 h. *P<0.01 vs. the LPA group; **P<0.01 vs. the control group. LPA, lysophosphatidic acid; TNF-α, tumor necrosis factor-α.

increased markedly within a short period of time following the addition of LPA to the T lymphocytes. The TNF-α concentration reached its maximum at 4 h and stabilized within 24 h (Table III; Fig. 1).

*Effects of LPA on voltage-dependent potassium ($K_v$) currents*. A total of 28 voltage-dependent $K^+$ currents of Jurkat T cells were obtained. To exclude the natural attenuation of $K_v$, $K_v$ was continuously observed for 30 min, which determined that no other current was significantly attenuated. A total of 7 Jurkat T cells were observed in the control group (205.5±43.4 pA; Fig. 2A), 7 Jurkat T cells were observed in the 0.5 $\mu$mol/l LPA group (246.65±30.9 pA; Fig. 2B), 7 Jurkat T cells were observed in the 5 $\mu$mol/l LPA group (317.5±32.1 pA; Fig. 2C), and 7 Jurkat T cells were observed in the 50 $\mu$mol/l LPA group (361.8±46.7 pA; Fig. 2D).

The $K_v$ current amplitude in the 5 $\mu$mol/l LPA group (317.5±32.1 pA) and the 50 $\mu$mol/l LPA group (361.8±46.7 pA) significantly increased compared with the control group (205.5±43.4 pA; P<0.01). The $K_v$ current amplitude in the 0.5 $\mu$mol/l LPA group (246.65±30.9 pA) was significantly increased compared with the control group (P<0.05). The effect of LPA on $K_v$ currents and the I-V curve of $K_v$ currents are shown in Fig. 2E. It was deduced that LPA had a dose-dependent effect on the $K_v$ current. The electrophysiological characteristics (current amplitude and activation, and inactivation voltage range) of $K_v$ in the present study were concordant with those of previous reports (14,15).

*Effects of LPA on $Ca^{2+}$-activated potassium ($K_{ca}$) currents*. The $K_{Ca}$ of Jurkat T cells in the 5 $\mu$mol/l LPA group and the 10 $\mu$mol/l Ki16425 + 5 $\mu$mol/l LPA group were obtained to observe the effects of LPA on $K_{Ca}$ and to examine the inhibitory effect of Ki16425. Ionic currents of $K_{Ca}$ were recorded under the various voltage-clamp protocols of the step pulse: Holding potential -80 mV, test potentials -80 to +80 mV, step 10 mv, and duration 400 ms. Compared with the current amplitude prior to treatment with LPA (439.6±43.7 pA; Fig. 3A), the $K_{Ca}$ current amplitude in the 5 $\mu$mol/l LPA group (628.5±46.1 pA; Fig. 3B) increased significantly (P<0.01). The $K_{Ca}$ current amplitude in the 10 $\mu$mol/l Ki16425 + 5 $\mu$mol/l LPA group decreased to 507.5±71.4 pA (Fig. 3C), which suggests that Ki16425 was able to inhibit the effect of LPA (Fig. 3).

*Effects of LPA on Cx43 protein expression*. The expression of Cx43 was determined by immunohistochemical SP staining. Cx43 was abundantly expressed in the control group and displayed marked positive staining. Cx43 was uniformly distributed in the intercalated disk between adjacent cells and had a clustered distribution (Fig. 4). Cx43 in the LPA + AMI model group was clearly decreased and had a disordered, uneven and punctate distribution (Fig. 5). The expression of Cx43 in the Ki16425 + LPA group was very similar to the control group (Fig. 6). The LPA + immune-suppressed group expressed decreased Cx43 protein compared with the LPA + AMI model group. Cx43 was distributed relatively uniformly in the intercalated disk between adjacent cells and had a dotted distribution; however, both the density and coloring level of Cx43 were decreased as compared with the control group (Fig. 7). The expression of Cx43 in the LPA + immune-enhanced group was marginal (Fig. 8).

## Discussion

LPA is an intermediate product of membrane phospholipid metabolism (1,4,12). Previous studies have demonstrated that

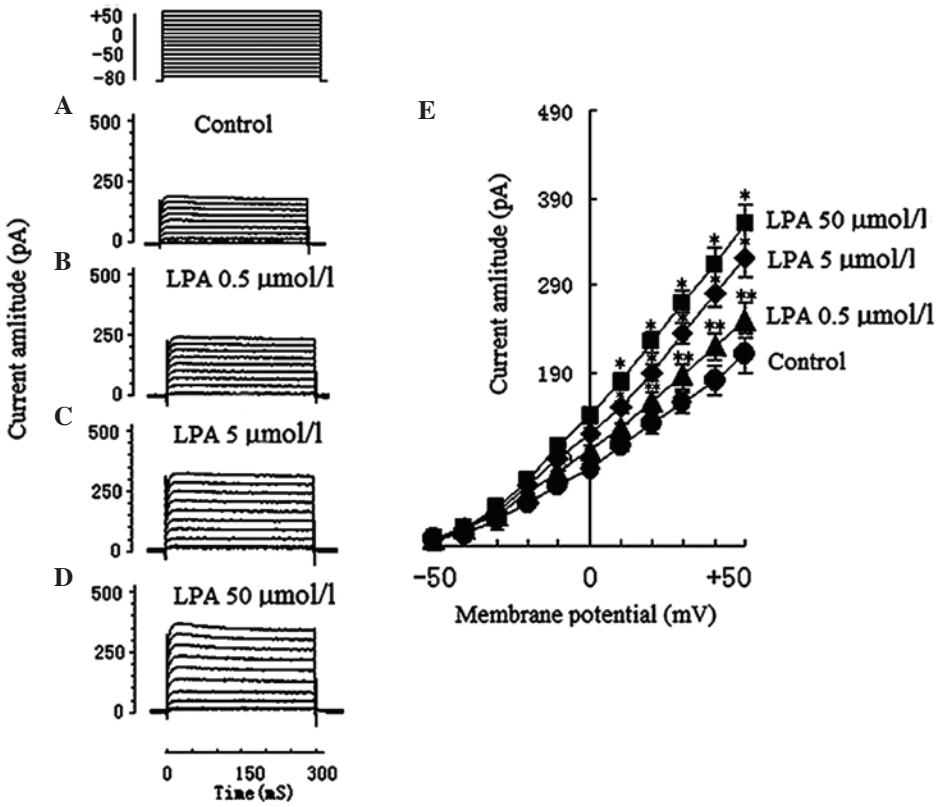


Figure 2. Effect of LPA on $K_v$ currents. Patch clamp records of the (A) control, (B) 0.5 $\mu$mol/l LPA, (C) 5 $\mu$mol/l LPA and (D) 50 $\mu$mol/l LPA groups. (E) $K_v$ current amplitude in the 5 $\mu$mol/l LPA (317.5±32.1 pA) and 50 $\mu$mol/l LPA groups (361.8±46.7 pA) significantly increased, as compared with the control group (205.5±43.4 pA; $^{*}$P<0.01). $K_v$ current amplitude in the 0.5 $\mu$mol/l LPA group (246.65±30.9 pA) was increased significantly, as compared with the control group ($^{**}$P<0.05). $K_v$, voltage-dependent potassium; LPA, lysophosphatidic acid.

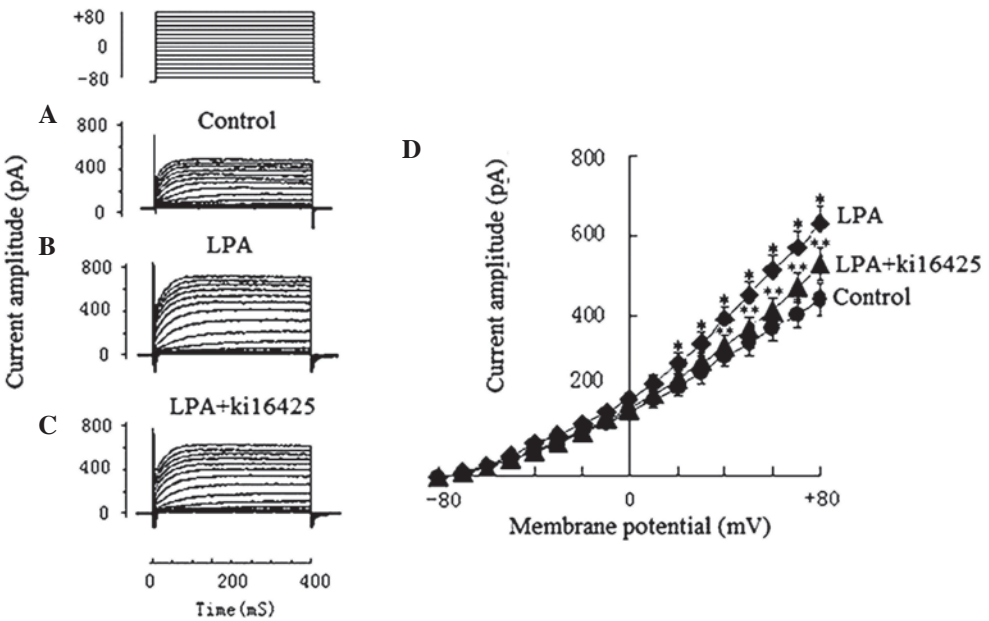


Figure 3. Effects of LPA on $K_{Ca}$ currents. Patch clamp records of the (A) control, (B) 5.0 $\mu$mol/l LPA, (C) and 5.0 $\mu$mol/l LPA + 10 $\mu$mol/l Ki16425 groups. (D) Current-voltage curve of $K_{Ca}$. $K_{Ca}$ current amplitude was significantly increased in the LPA group (628.5±46.1 pA), as compared with the control group (439.6±43.7 pA; $^{*}$P<0.01). $K_{Ca}$ current amplitude was significantly decreased in the LPA + Ki16425 group, as compared with the LPA group ($^{**}$P<0.001), demonstrating that Ki16425 was able to block the effect of LPA. $K_{Ca}$, calcium-activated potassium; LPA, lysophosphatidic acid.

LPA has an important role in cardiovascular disease. LPA levels increase in infarcted myocardium following AMI (4,16). In addition, LPA release by platelets simultaneously increases, resulting in markedly increased LPA concentration levels in regional myocardial tissue and plasma (17,18). Leukocyte concentrations increase in the peripheral blood of patients with

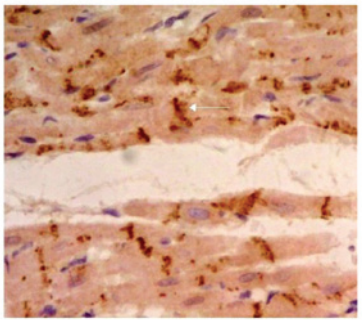

Figure 4. Cx43 expression in the control group. The expression of Cx43 was determined by immunohistochemical SP staining. Cx43 was abundantly expressed in the control group and displayed strong positive staining, as identified by the white arrow. Magnification, x400. Cx43, connexin 43.

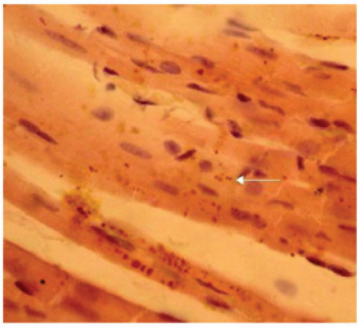

Figure 5. Cx43 expression in the LPA + AMI model group. Cx43 expression in the LPA + AMI model group markedly decreased and exhibited a disordered, uneven and punctate distribution, as identified by the white arrow. Magnification, x400. Cx43, connexin 43; LPA, lysophosphatidic acid; AMI, acute myocardial infarction.

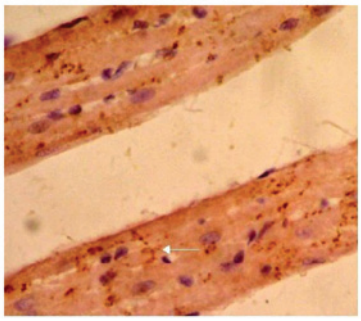

Figure 6. Cx43 expression in the Ki16425 + LPA group. Cx43 expression in the Ki16425 + LPA group was similar to the control group, as identified by the white arrow. Magnification, x400. Cx43, connexin 43; LPA, lysophosphatidic acid.

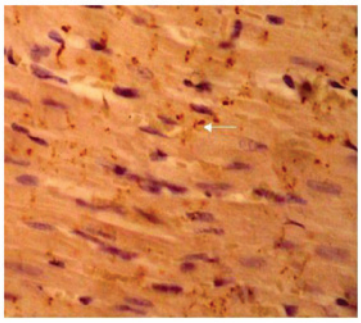

Figure 7. Cx43 was abundantly expressed in the LPA + immune-suppressed group, as identified by the white arrow. Magnification, x400. Cx43, connexin 43; LPA, lysophosphatidic acid.

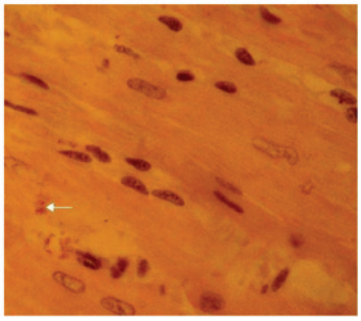

Figure 8. Expression of Cx43 in the LPA + immune-enhanced group was rare, as identified by the white arrow. Magnification, x400. Cx43, connexin 43; LPA, lysophosphatidic acid.

AMI, and inflammatory cells infiltrate the coronary arteries surrounding the tissue (19). These results suggests that AMI is a process of the immune inflammatory response. Xie *et al* (20) and Okudaira *et al* (21) hypothesized that LPA may modulate immune inflammatory responses. In the present study, we hypothesized that LPA, acting as an important immune regulatory substance, may be able to induce the release of various cytokines through the activation of certain immune cells, thereby inducing arrhythmia.

Immune-enhanced and immune-suppressed rat models were constructed to validate the hypothesis. By observing the surface ECGs of AMI rats, the results demonstrated that the pro-arrhythmic effect of LPA was closely associated with immune status. The incidence of arrhythmia decreased when the immune systems of the rats were suppressed. To exclude interference by *in vivo* factors and to examine the effect of LPA on arrhythmia, LPA was added to the perfusate of isolated hearts from rats of the immune-enhanced and the immune-suppressed groups. The results demonstrated that the isolated rat hearts of the immune-enhanced group exhibited increased occurrences of VPBs compared with normal rats and rats of the immune-suppressed group. This suggested that LPA has a role in the occurrence of arrhythmia and is closely associated with the rat immune status.

Various cytokines are closely associated with arrhythmia, including TNF, IL-6, IL-8 and IL-10 (19,22,23). TNF is considered to be one of the most important cytokines in ischemia/reperfusion injury in patients with AMI (24,25). TNF-α is a multifunctional cytokine; as a key inflammatory mediator of AMI, TNF-α has been the subject of increased research. Animals overexpressing TNF suffer from severe heart disease, including arrhythmia (26-28). This indicates that upregulation of TNF expression is closely associated with the dysfunction of cardiac myocytes (29,30). The results of the present study demonstrated that LPA is able to induce TNF-α release in cultured Jurkat T cells. These data suggest that LPA induces TNF-α mRNA expression and promotes the synthesis and release of TNF-α by activating Jurkat T cells.

Lymphocyte activation is closely associated with $K^+$ channels on the lymphocyte membrane. $K^+$ channels function in controlling membrane potential, regulating cell volume and activating lymphocytes (31,32); therefore, they have an important role in the process of lymphocyte immunity. The present study investigated the current characteristics of voltage-dependent $K^+$ channels ($K_v$) and $Ca^{2+}$-activated $K^+$ channels ($K_{Ca}$) in Jurkat T cells. The results demonstrated that LPA increased the $K_v$ current in Jurkat T cells and promoted the influx of $K^+$. LPA significantly increased the current amplitude of $K_{Ca}$. These results provide further evidence that LPA activates Jurkat T cells by opening $K_v$ and $K_{Ca}$ channels.

The rhythmic contraction of the heart is dependent on signal transduction between myocardial cells (33,34). It has been demonstrated that gap junctions (GJs) are the primary mode of signal transduction between cells (35,36). GJs are predominantly composed of connexin, and Cx43 is the main protein of GJs in the heart (37-39). The incidence of arrhythmia increased significantly and Cx43 decreased when LPA expression was upregulated (40,41). We hypothesize that LPA affects signal transduction between myocardial cells by suppressing the synthesis and expression of Cx43, thereby causing arrhythmia. The expression pattern of Cx43 was observed in the myocardium using immunohistochemical staining. The results indicated that LPA caused the degradation of Cx43 and decreased the expression of Cx43. This may be one of the most important mechanisms underlying the regulation of LPA-induced arrhythmias. The observation that LPA caused the degradation of Cx43 and decreased its expression is relevant to the immune status of rats.

In summary, the results of the present investigation determined that LPA participates in the incidence of arrhythmia following AMI. To the best of our knowledge, the results provide the first experimental evidence that LPA causes arrhythmia via the regulation of immune-inflammatory cells and the release of cytokines. The experiments demonstrate that LPA is able to induce TNF-α expression by activating T lymphocytes and suppressing the synthesis and expression of Cx43.

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