THE ANATOLIAN JOURNAL OF CARDIOLOGY

Official journal of the TURKISH SOCIETY OF CARDIOLOGY

The Effects of Lipopolysaccharide Derivatives in Rodent Models of Cardiac Arrhythmia

ORIGINAL INVESTIGATION

ABSTRACT

Background: Several previous studies have suggested that sublethal doses of *Escherichia coli* lipopolysaccharide (endotoxin) and monophosphoryl lipid A Re595, a non-pyrogenic derivative of *Salmonella minnesota* lipopolysaccharide, exhibit antiarrhythmic effects in the rat model of ischemia–reperfusion arrhythmias.

Methods: In this study, the protective effect of lipopolysaccharide derivatives was also further investigated in drug (aconitine or ouabain)-induced arrhythmia models, and conclusions were drawn with particular emphasis on the molecular characteristics of different types of lipopolysaccharide.

Results: The importance of the molecular structure for the antiarrhythmic effect of monophosphoryl lipid A and *E. coli* lipopolysaccharide was tested in the ischemia–reperfusion arrhythmia model. In contrast to monophosphoryl lipid A from *Salmonella typhimurium* SL 684 which has only monophosphoryl residue in its structure, monophosphoryl lipid A Re595, obtained from *S. minnesota*, and *E. coli* lipopolysaccharide which have both mono and diphosphoryl residue reduced the duration of ventricular tachycardia (e.g., during reperfusion: vehicle: 176 ± 22.8; monophosphoryl lipid A Re595: 132.83 ± 12.1, as second, n = 8-10, $P < .05$) and the incidence of ventricular fibrillation.

The antiarrhythmic effects of *E. coli* lipopolysaccharide and monophosphoryl lipid A Re595 in ischemia–reperfusion arrhythmia model were absent in either aconitine- (e.g., onset time for ventricular ectopic beats: saline 25.3 5.0, *E. coli* lipopolysaccharide 24.3 ± 7.1; vehicle: 24.0 ± 4.5, monophosphoryl lipid A SL684 23.8 ± 4.3, as second, n = 6, $P > .05$) or ouabain-induced arrhythmia models in mice.

Conclusion: Therefore, we conclude that lipopolysaccharide derivatives exhibit antiarrhythmic effect only in ischemia–reperfusion arrhythmias, and lipopolysaccharide should possess diphosphoryl groups in its subcomponent composition for this antiarrhythmic effect.

Keywords: Arrhythmia, aconitine, ouabain, monophosphoryl lipid A, endotoxin, lipopolysaccharide, prophylaxis

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Received: January 24, 2022
Accepted: July 5, 2022
Available Online Date: July 21, 2022

Cite this article as: Yilmaz G, Boz M, Iskit AB. The effects of lipopolysaccharide derivatives in rodent models of cardiac arrhythmia. *Anatol J Cardiol*. 2022;26(12):886-892.

DOI:10.5152/AnatolJCardiol.2022.1524

INTRODUCTION

We have previously demonstrated that sublethal doses of Gram-negative bacterial lipopolysaccharides (LPS, endotoxin) markedly reduced the severity of ischemia–reperfusion arrhythmias. The mechanism appears to involve nitric oxide production through the activation of inducible nitric oxide synthase.[1] In addition, an LPS derivative monophosphoryl lipid A (MLA), like LPS itself, also reduces the severity of life-threatening arrhythmias which result from coronary artery occlusion and reperfusion.[2] Although responses to either LPS or MLA administration involve multiple factors,[3] particular interest has focused on the possibility that nitric oxide might have a major role in several studies.[2,4]

Monophosphoryl lipid A is a non-toxic derivative of lipid A component of LPS molecule from Gram-negative bacteria such as *Salmonella typhimurium*, *Salmonella minnesota R595,* or *Chlamydia trachomatis* that lack many of the endotoxic properties of the parent molecule. Monophosphoryl lipid A was extracted to reduce the associated toxicity while retaining the immunomodulatory properties of the parent LPS molecule such as macrophage stimulation and cytokine

release.[5] Monophosphoryl lipid A has also been shown to contain some of the beneficial properties of LPS: MLA induces myocardial protection against ischemia–reperfusion induced infarction, arrhythmia, and myocardial stunning in ischemia-reperfusion arrhythmia models of mice,[5] rats,[2] rabbits,[6] and dogs.[7] This effect was associated with increased myocardial catalase activity only if MLA was administered 24 hours, not 1 hour before coronary artery occlusion of canine hearts.[7] Some reports indicate that the phosphates attached to lipid A may also play an essential role in determining its bioactivity. There is no satisfactory investigation into the structure–function relationship of MLA types.[8] To this point, the beneficial effect of MLA on myocardium was investigated only with *Salmonella minnesota* Re 595 (R mutants; MLA595) which has both "mono and diphosphoryl" residues in its structure.[2] However, there is no knowledge about the effects of MLA obtained from *Salmonella typhimurium* SL684 (Rc mutant; MLA684) which has a different structure: only monophosphoryl residue.

It has been suggested that during myocardial ischemia, there is a loss of intracellular K^+ from jeopardized cells and a nonuniform shortening of the action potential which is responsible for life-threatening ventricular arrhythmias. However, according to the literature, we do not have any knowledge about the effects of LPS and LPS derivatives on different kinds of arrhythmia models such as aconitine, related to sodium current (I_{Na};[9]) and ouabain, related to Ca^{2+} overload,[10] which are used as a screening test for antiarrhythmic drug discovery in mice.[11,12]

Therefore, this study aims to investigate the underlying mechanisms of LPS-induced antiarrhythmic effect by using different types of experimental arrhythmia models and to determine the subcomponent composition which is necessary and/or responsible for the antiarrhythmic effects of LPS and its derivative MLA.

METHODS

Animals

Fifty-six male Sprague–Dawley rats (200-300 g) and 60 male Swiss albino mice (20-28 g) were obtained from the Laboratory Animal Husbandry Facility of the Department of Pharmacology, Hacettepe University Faculty of Medicine, and were housed in environmentally controlled conditions at 21 ± 2°C and 30%-70% relative humidity with 12-hour dark/12-hour light illumination sequence (the lights were on between 7:00 AM and 7:00 PM) with *ad libitum* access to tap water (drinking bottle) and standard pellet dairy chow (Murat Yem Sanayi, Ankara, Turkey). The Guiding Principles in the Care and Use of Laboratory Animals together with The Recommendations from the Declaration of Helsinki were strictly adhered to during the execution of all the procedures described within this manuscript. This project was approved by the institutional Experimental Animal Care and Use Ethics Committee of Hacettepe University before the commencement of any intervention.

Drug Administration

In aconitine and ouabain arrhythmia models, LPS derived from *Escherichia Coli* (O55:B5; 1 mg kg^{-1}) or an equivalent volume (1 mL kg^{-1}) of non-pyrogenic sterile saline (NaCl 0.9%, w/v, dissolved in pyrogen-free distilled water) was given by intraperitoneal injection to mice 4 hours before the experiment. Monophosphoryl lipid A (*S. minnesota* Re595; 5 mg kg^{-1}, intraperitoneal (i.p.)) or an equivalent volume of its vehicle (40% propylene glycol, 10% ethanol, and 50% water for injection) was given intraperitoneally to mice 24 hours before the experiment. The dose and the timing of LPS and MLA administration were chosen based on previous studies in which the antiarrhythmic effects were observed.[1,2]

In ischemia–reperfusion arrhythmia model, MLA derived from *Salmonella typhimurium* (SL684, Rc mutant; 5 mg kg^{-1}), MLA derived from *Salmonella minnesota* Re595; 5 mg kg^{-1}, i.p.) or an equivalent volume (0.1 ml kg^{-1}) of its vehicle (40% propylene glycol, 10% ethanol, 50% water for injection) was given by intraperitoneal injection to rats 24 hours before the experiment. Also, LPS derived from *E. coli* (O55:B5) or an equivalent volume (0.1 ml kg^{-1}) of non-pyrogenic sterile saline (NaCl 0.9 %, w/v, dissolved in pyrogen-free distilled water) was given by i.p. injection to rats 4 hours before they underwent surgery. The timing of LPS and MLA administration was chosen based on previous studies in which the antiarrhythmic effect was observed.[1,2] Control animals received an equal volume (1 mL kg^{-1}, i.p.) of saline.

All drugs were prepared daily, dissolved in non-pyrogenic sterile saline, and warmed appropriately to the body temperature (37°C) before injection. Drug solutions were kept in the dark containers until injected to protect them from light-induced decomposition.

Surgical Procedure

Aconitine- and Ouabain-Induced Arrhythmias in Mice

The method for aconitine- and ouabain-induced arrhythmias was adapted from the literature.[10-12] Briefly, mice were anesthetized with sodium thiopental (65 mg kg^{-1}, i.p.) and placed on a heat-insulated-cork sheet-covered operating table. Heart rate was monitored and displayed on Harvard

HIGHLIGHTS

- Sublethal doses of Gram-negative bacterial endotoxin (lipopolysaccharides) markedly reduce the severity of ischemia–reperfusion arrhythmias.
- Despite having a considerable antiarrhythmic effect, lipopolysaccharide cannot be employed as a medication due to its severe toxicity.
- A safe and well-tolerated lipopolysaccharide derivative monophosphoryl lipid A also reduces the severity of life-threatening arrhythmias which result from coronary artery occlusion and reperfusion.
- Lipopolysaccharide derivatives are antiarrhythmic only in ischemia–reperfusion models, and it was determined that diphosphoryl groups, subcomponent composition, are necessary for the antiarrhythmic effect.

Oscillograph pen recorder together with a standard lead-II electrocardiogram (ECG). Aconitine (100 mg kg^{-1}) or ouabain (15 mg kg^{-1}) was administered intravenously from the tail vein. The arrhythmogenic agents' dose was chosen based on previous studies.[9,11]

The onset of arrhythmia or ventricular tachycardia (VT) was accepted as the time of the occurrence of the first extrasystole or the onset of VT was recorded. The body temperature of the animals (37°C) was stabilized within the 0.1°C limits by a rectal thermistor probe-controlled incandescent lamp.

Coronary Artery Ligation in Rats

Fundamental surgical procedures used in the present study were described previously by Clark et al[13] and Iskit et al.[14-16] Briefly, rats (200-300 g) were anesthetized with sodium thiopental (65 mg kg^{-1}, i.p.) and placed on a heat-insulated-cork sheet-covered operating table. The trachea and left jugular vein were cannulated for artificial respiration and drug administration. Arterial blood pressure was monitored from the left carotid artery by using PX23L pressure transducer (Statham, Hato Rey, Puerto Rico) and displayed on the Harvard Oscillograph (Harvard Apparatus, Holliston, Massachusetts, United States) pen recorder together with a standard lead-II ECG. The chest was opened by a left thoracotomy, followed by sectioning of the fourth and fifth ribs, 2 mm to the left of the sternum. Positive-pressure artificial respiration with Small Animal Respirator (Cole-Parmer, Vernon Hills, Illinois, USA; volumetrically calibrated according to the displacement of water column inside an inverted measure immersed in a water tank) was started immediately with room air, using a volume of 1.5 mL per 100 g body weight at a rate of 60 strokes per minute. Body temperatures of the rats (37°C) were stabilized within the 0.1°C limits by a rectal thermistor probe-controlled incandescent lamp placed 30 cm above the abdominal region.

After the pericardium was incised, the heart was eased out of the chest by gentle pressure on the rib cage. Next, a 6/0 silk suture, attached to a 10-mm micro point reverse-cutting needle was placed under the left main coronary artery. When the heart was replaced in the chest, attention was paid to the reinflation of the lungs properly, and the animal was allowed to recover for a stabilization period of 15 minutes.

Just before the occlusion of the left coronary artery, 0.1 mL of arterial blood sample was withdrawn to a heparin-coated (flushed twice with 100 IU mL^{-1} of heparin) 1 mL syringe. In saline-treated animals, blood gas analysis was performed by using AVL Type 995 (Roche, Rotkreuz, Switzerland) blood gas autoanalyzer at 37°C. At the same time, the results were corrected automatically according to the air pressure (around 690 mm Hg in the laboratory) at the time of the analysis. The artificial respiration parameters mentioned above yielded arterial PaO_2 values always greater than 70 mm Hg, $PaCO_2$ values were always lower than 40 mm Hg, pH values were between 7.35 and 7.45, oxygen saturation was around 95%, and HCO_3 values was around 20 mmol L^{-1}.

Any animal in which this surgical procedure produced arrhythmias or a sustained decrease in mean arterial blood pressure below 60 mm Hg during the stabilization period was discarded. A small plastic snare was threaded through the ligature and placed in contact with the heart. The artery could be occluded by applying tension to the ligature, and the reperfusion was achieved by releasing the tension. After the equilibration period of 15 minutes, the artery was occluded for 7 minutes and reperfused for another 7 minutes. This experimental protocol was chosen since we are familiar with its outcome.[14-16]

Evaluation of Arrhythmias

Electrocardiogram and arterial blood pressure recordings were monitored throughout the occlusion and reperfusion periods, and the heart rate was derived from these recordings. The ventricular ectopic activity was assessed according to the diagnostic and analytic criteria advocated in Lambeth Conventions.[17] No effort was made to reanimate or to defibrillate any animal that exhibited fatal arrhythmias. The duration (in seconds) of VT was quantified only in survivors. The incidences of VT, ventricular fibrillation (VF), and mortality were quantified for both periods. In aconitine- and ouabain-induced arhythmia models, the first ventricular ectopic beat (VEB) and VT's onset time was measured from the ECG.

Drugs Used

Sodium chloride (Merck, Burlington, Massachusetts, USA), sodium thiopental (I. E. Ulagay, İstanbul, Turkey), heparin sodium (Roche, Basel, Switzerland), LPS (*E. coli* endotoxin, serotype 055:B5, Sigma, St. Louis, Missouri, USA), MLA (*S. typhimurium* SL684, Rc mutant, Sigma), MLA (*S. minnesota* Re 595, Sigma), aconitine (Sigma), and ouabain (Sigma) are the drugs used.

Statistical Analysis

Statistical analysis was performed using the GraphPad Prism software (GraphPad Software Inc., Calif, USA). Normally distrubuted data are expressed as means ± standard error of the mean of the number (n) of experiments or median interquartile range (25%-75%) owing to their non-normal distribution. The normal distribution suitability of the groups was assessed using the Shapiro-Wilk test. Two-way analysis of variance for repeated measurements was performed to analyze the differences between the mean arterial blood pressure curves plotted over time. Student's *t*-test was used for single-time point evaluation for blood pressure (independent, unpaired). Differences between the medians were analyzed using Mann–Whitney *U*-test or Kruskal–Wallis test followed by Dunn's post-test (e.g., onset time for VEB and VT duration). In addition, Fischer's exact test was used to analyze the differences between the VF incidences of arrhythmias and mortality. When $P < .05$, the differences were accepted as being statistically significant.

RESULTS

Fifty-six rats and 60 mice underwent surgical preparation for the experiments and were included in this study. Satisfactory anesthesia was achieved as judged by the obtundation of responses to a painful stimulus (i.e., pinching the foot with

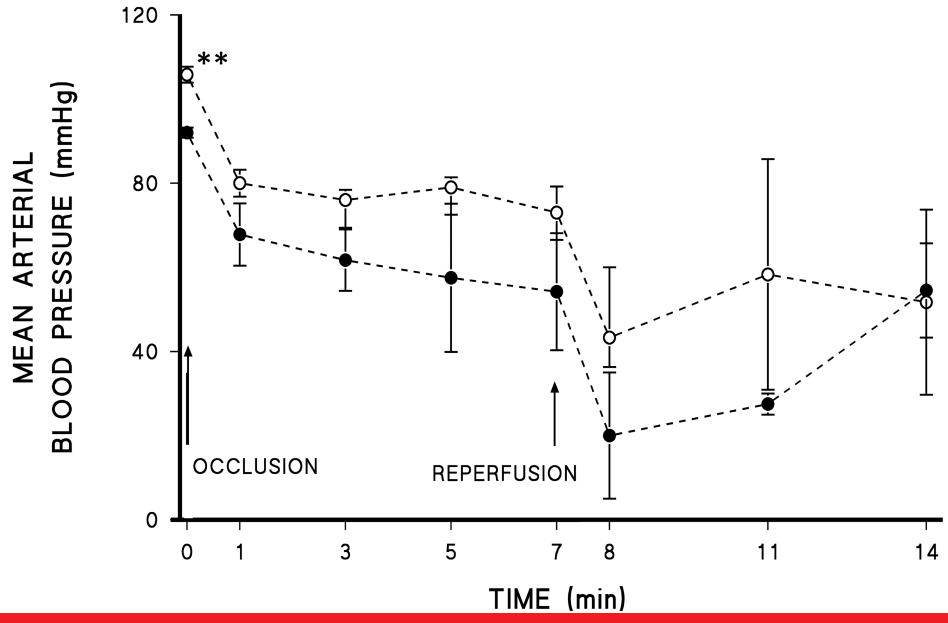

Figure 1. Mean arterial blood pressure (mm Hg) values plotted against time in saline- (○- - -○) and LPS (●- - -●; *Escherichia coli* endotoxin, serotype 055:B5, 1 mg kg^{-1})-treated rats under sodium thiopental (65 mg kg^{-1}, i.p.) anesthesia undergoing coronary artery occlusion and reperfusion at time points indicated by the arrows. Vertical bars indicate the standard error of the mean of the number of data points included in that observation. n = 8-12 for each data point obtained from the survivors for that period. $P > .05$ for saline vs. LPS curves (ANOVA for repeated measures), $P < .05$ for initial mean arterial blood pressure values (Student's *t*-test independent, unpaired). ANOVA, analysis of variance; LPS, lipopolysaccharide.

tweezers). None of the drugs significantly modified the heart rate of the mice and rats (around 480 ± 20 and 420 ± 25 beats per minute).

Coronary Artery Ligation in Rats

The Effects of Monophosphoryl Lipid A SL684, Re595, and Lipopolysaccharide on the Blood Pressure

Figure 1 shows the mean arterial blood pressure curves obtained from control and LPS (1 mg kg^{-1})-treated groups. Coronary artery occlusion resulted in a significant fall in the mean arterial blood pressure which returned to baseline values toward the end of the reperfusion period, and curves obtained from either anesthesia group were almost superimposable when plotted against time (n = 8-12, $P > .05$; Figure 1). There was only a difference between the initial mean arterial blood pressure values and curves of the 2 groups (at 0 minute, mm Hg, vehicle: 110.8 ± 1.9, n = 10; LPS: 89 ± 1.2, n = 12, $P < .01$; Figure 1). Neither MLA SL684 nor MLA Re595 exhibited the vehicle's initial mean arterial blood pressure value (data not shown).

The Effects of Lipopolysaccharide, Monophosphoryl Lipid A SL684, and Monophosphoryl Lipid A Re595 on Ventricular Tachycardia Duration, Ventricular Fibrillation Incidence, and Mortality

Table 1 summarizes the duration of VT, the incidence of VF, and total mortality observed in LPS- or MLA-administered rats. Lipopolysaccharide and MLA Re595 also exerted an antiarrhythmic effect and decreased mortality both during myocardial ischemia and reperfusion.

In LPS-treated rats, the durations of VT were significantly shorter during both occlusion and reperfusion periods (n = 8-12,

Table 1. Duration of VT Together with the Incidences of VF and Mortality in Saline or LPS (Endotoxin) and in Vehicle or MLA SL684, Re 595-Treated Rats Subjected to Coronary Artery Occlusion and Reperfusion.

	Occlusion			Reperfusion		
Groups	**VT Duration (s)**	**VF Incidence (%)**	**Mortality (%)**	**VT Duration (s)**	**VF Incidence (%)**	**Mortality (%)**
Saline	39.6 ± 13.5 (n = 10)	0/10 (0%)	2/10 (20%)	184 ± 52.3 (n = 8)	8/8 (100%)	4/8 (50%)
LPS	0** (n = 12)	0/12 (0%)	0/12* (0%)	152.8 ± 22.1* (n = 12)	9/12* (75%)	4/12* (33%)
Vehicle	37.6 ± 16.1 (n = 10)	0/10 (0%)	2/10 (20%)	176 ± 22.8 (n = 8)	8/8 (100%)	4/8 (50%)
MLA 684	35.5 ± 10.1 (n = 12)	0/12 (0%)	2/12 (17%)	162.8 ± 32.1 (n = 10)	7/8 (88%)	6/12 (50%)
MLA 595	14.3 ± 8.1* (n = 12)	0/12 (0%)	0/12* (0%)	132.8 ± 12.1* (n = 10)	6/8* (75%)	4/12* (33%)

* Indicates $P < .05$ vs. adjacent saline/vehicle, ** indicates $P < .01$ vs. saline (Mann–Whitney *U* test or Kruskal–Wallis test followed by Dunn's post-test for VT duration; Fischer's exact test for VF incidences and mortality). VF, ventricular fibrillation; VT, ventricular tachycardia; MLA, monophosphoryl lipid A; LPS, lipopolysaccharide.

Table 2. Onset Time of Ventricular Ectopic Beat and Ventricular Tachycardia in Saline or LPS (Endotoxin), Vehicle, or *Salmonella typhimurium* SL684 (Rc mutant; MLA 684)- and *Salmonella minnesota* Re 595 (R mutants; MLA 595)-Treated (5 mg kg^{-1}) mice

	Aconitine-Induced Arrhythmia Onset Time as Second		Ouabain-Induced Arrhythmia Onset Time as Second	
	VEB	VT	VEB	VT
Saline	25.3 ± 5.0 (n = 6)	41.3 ± 4.1 (n = 6)	18.5 ± 3.9 (n = 6)	38.0 ± 2.6 (n = 6)
Endotoxin	24.3 ± 7.1 (n = 6)	47.4 ± 15.3 (n = 6)	19.3 ± 6.1 (n = 6)	56.3 ± 18.2 (n = 6)
Vehicle	24.0 ± 4.5 (n = 6)	40.5 ± 2.5 (n = 6)	18.0 ± 3.2 (n = 6)	45.3 ± 4.8 (n = 6)
MLA 684	22.8 ± 5.1 (n = 6)	44.7 ± 12.0 (n = 6)	19.0 ± 3.7 (n = 6)	54.7 ± 14.2 (n = 6)
MLA 595	23.8 ± 4.3 (n = 6)	44.2 ± 8.0 (n = 6)	17.0 ± 2.4 (n = 6)	52.8 ± 4.3 (n = 6)

P > .05 (Mann–Whitney *U* test or Kruskal–Wallis test). VEB, ventricular ectopic beat; VT, ventricular tachycardia; MLA, monophosphoryl lipid A; LPS, lipopolysaccharide.

saline vs. LPS; *P* < .01 and *P* < .05 respectively). A decline in mortality rates was also observed (*P* < .05). Similiar to LPS, MLA 595 treatment also shortened the durations of VT during both occlusion and reperfusion periods (n = 8-12, vehicle vs. LPS; *P* < .05). A decline in mortality rates was also observed (*P* < .05).

However, none of the parameters were significantly different from its corresponding control value (vehicle) for MLA SL684, and the antiarrhythmic effect was not observed (*P* > .05; Table 1).

Aconitine- and Ouabain-Induced Arrhythmias in Mice

The Effects of Endotoxin on the Onset Time in Aconitine- and Ouabain-Induced Arrhythmias

There was no difference between the onset time of saline- and endotoxin-treated mice in aconitine-or ouabain-induced arrhythmias (first 2 rows, *P* > .05; Table 2).

The Effects of Monophosphoryl Lipid A Re595 and Monophosphoryl Lipid A SL684 on the Onset Time in Aconitine- and Ouabain-Induced Arrhythmias

There was no difference between the onset time of vehicle- and MLA Re595-treated mice in aconitine- or ouabain-induced arrhythmias (last 3 rows, *P* > .05; Table 2).

DISCUSSION

Lipopolysaccharide (endotoxin) derived from *E. coli* protects the heart against life-threatening ventricular arrhythmias during ischemia and reperfusion.[1,4] In this study, the antiarrhythmic effect of *E. coli* LPS or MLA Re595 *in vivo* ischemia–reperfusion arrhythmias[1,6] was not observed in drug-induced arrhythmia models, and MLA SL684, in contrast to MLA Re595, did not attenuate ischemia–reperfusion arrhythmias.

For many years, it has been known that endotoxin has profound effects on the heart such as depression of the myocardial contractility[18-20] and cardiac responses to exogenous catecholamines *in vivo*.[21] Enhanced antioxidant status with increased endogenous myocardial catalase activity and overexpression of superoxide dismutase after endotoxin exposure were reported to decrease ischemia–reperfusion injury of dog hearts *in vivo*.[7] Also, various cardioprotective mediators like heat shock proteins, some phosphodiesterase isoforms, Na^+/K^+ ATPase, and nitric oxide have been triggered by endotoxin.[22-24] Among various mediators, particular interest has focused on nitric oxide which has also been implicated in the antiarrhythmic and cardioprotective effects of preconditioning against ischemia and can also be inhibited by dexamethasone.[25,26] Despite the fact that L-NAME, a nitric oxide synthase inhibitor that inhibits both the constitutive (cNOS) and inducible (iNOS) versions of the enzyme, it failed to change the number or severity of ischemia or reperfusion arrhythmias in anesthetized rats.[14] In previous studies, by using intracellular recording methods, it was shown that atria obtained from LPS-treated rats had prolonged action potential duration. The prolongation of myocardial action potential duration could be either due to increased Ca^{2+} channel activity during the plateau phase or blockade of any of the K^+ channels.[16]

Ouabain, a cardiac glycoside, inhibits Na^+/K^+ ATPase and raises intracellular Na^+ concentration. The rise in intracellular Na^+ concentration causes intracellular Ca^{2+} overload due to activation of sarcolemmal Na^+/Ca^{++} exchange. The Ca^{2+} overload, in turn, induces oscillatory Ca^{2+} release from the sarcoplasmic reticulum and oscillatory fluctuation in resting potential. An ionic current associated with this Ca^{2+} oscillation has been named a transient inward current (TI). The oscillatory Ca^{2+} rise by TI induces the enhancement of systolic (positive inotropic effect) and diastolic tension (positive tonotropic effect) and the extra beatings (arrhythmias).[10,27,28] During this study, different from ischemia–reperfusion arrhythmia models,[1] endotoxin, MLA Re595, and MLA SL684 did not modify arrhythmia parameters (such as the onset of initial VEB and VT) in oubain-induced arrhythmia model. These results suggest that the antiarrhythmic effect of LPS and MLA Re595 in ischemia–reperfusion arrhythmia model is independent of Ca^{2+} currents in the heart.

Aconitine, a highly toxic and arrhythmogenic alkaloid, produces arrhythmia when a toxic dose is administered and is used to induce arrhythmia in experimental animals.[9,12,29-31] Aconitine-induced arrhythmias are characterized by early afterdepolarizations, caused by increased residual I_{Na} during the plateau, and includes ventricular and supraventricular polytopic extrasystoles, bigeminy, and paroxysmal VT.[9] Aconitine also shifts the activation of sodium channels toward more negative membrane potentials in single heart muscle cells in mice.[32,33]

Different from ischemia–reperfusion arrhythmia models,[1] LPS, MLA Re 595, and MLA SL684 did not modify the onset of initial VEB and VT in aconitine- and ouabain-induced arrhythmia model. This result reveals that the antiarrhythmic effect of LPS and MLA Re595 in ischemia–reperfusion arrhythmia model is independent of Na^+ channels and Ca^{2+}

overload in the heart. This observation suggests the blockade of K^+ channels in the antiarrhytmic effect of LPS and its derivatives.

Lipid A is the toxic part of the LPS of Gram-negative bacteria eliciting a wide spectrum of pathophysiologic effects produced by stimulation of several host cells. The beneficial effects of MLA have been used in inducing tolerance to endotoxemia in both laboratory animals and humans, in immunotherapy or immunoprophylaxis, as an adjuvant for many vaccines, in inducing delayed protection against cerebral vasospasm caused by subarachnoid hemorrhage, and for delayed cardioprotection through complex mechanisms.[26] In the present study, MLA (*S. typhimurium* SL684, Rc mutant) containing "monophosphoryl residue" did not attenuate ischemia–reperfusion arrhythmias. Monophosphoryl lipid A used in this and previous studies had both "the mono and the diphosphoryl residues" (Re595 R mutant).[8,26] In this study, it was determined that diphosphoryl groups, subcomponent composition, were necessary or responsible for the antiarrhythmic effect of MLA. The antiarrhythmic effect of both LPS and MLA is explained with iNOS activation,[1,2] and diphosphoryl residue is necessary for this antiarrhythmic effect. It was previously demonstrated that the phosphate groups in the lipid A molecule also play important roles in human platelet activation via the protein kinase C pathway.[8] Therefore, it is possible that these monophosphoryl and diphosphoryl residues may have an important role in the activation of iNOS.

Monophosphoryl lipid A is a derivative of the LPS from Gram-negative bacteria which is safe and well-tolerated. It was extracted to reduce the associated toxicity while retaining the immunomodulatory properties of the parent LPS molecule. The extraction process decreases systemic toxicity by >99% compared to native lipid A, resulting in an immunomodulatory agent with greater potential for clinical use. In animal models, pretreatment with MLA enhances survival following otherwise deadly exposure to LPS. Monophosphoryl lipid A's positive benefits have also been used in cardioprotection, which uses complicated processes to prevent ischemia–reperfusion arrhythmias.[26] Monophosphoryl lipid A could be a promising candidate for prophylaxis of arrhythmias after myocardial infarction.

This study indicates that LPS derivatives are antiarrhythmic only in ischemia–reperfusion models. It was determined that diphosphoryl groups, subcomponent composition, are necessary or responsible for the antiarrhythmic effect of MLA. These results are precious and significant taken together with the known positive data of ours and others.[1,4] The current study fills an important gap in understanding the complete picture of LPS effects.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Hacettepe University (approval no: 99/99-29).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – G.Y., A.B.I.; Design – G.Y., A.B.I.; Supervision – A.B.I.; Funding – G.Y., A.B.I.; Materials – G.Y., A.B.I.; Data Collection and/or Processing – G.Y., M.B.; Analysis and/or Interpretation – M.B., A.B.I.; Literature Review – G.Y., M.B.; Writing – A.B.I.; Critical Review – A.B.I.

Acknowledgments: The authors are grateful to Prof. Dr. Erdem Karabulut, Department of Biostatistics, Faculty of Medicine, Hacettepe University (Turkey) for his invaluable advice on statistical analyses.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: This work is supported by Eczacibasi Scientific Research and Award Fund, Istanbul, Turkey and, partially by Hacettepe University Research Foundation (Project Number: 00.01.101.002).

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