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# XMU-MP-1 A attenuates myocardial depression following endotoxemia in hyperlipidemic mice model

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## Abstract

Endotoxic shock is a type of systemic inflammatory response, and many of the patients in the endotoxemic state are hypolipidemically ill or with hyperlipidemia. The main factor of endotoxemia is due to infection with pathogenic microorganisms. The main symptom is the level of endotoxins, which are one of the components of the outer wall of Gram-negative bacteria in the blood, increased significantly. With the development, it will induce a series of pathological processes in the body, such as myocardial damage. To study the effect of LPS on myocardial function, an ALI model was established in hyperlipidemic mice. C57BL/6J male mice weighing 18-22 g were selected to establish a hyperlipidemia model according to the literature. A total of 24 mice were selected and randomized into 4 groups (n = 6), in which the 23 mice required for modeling were at least 3 mice. After the model was successfully established, LPS was injected to establish the ALI. For drug administration, XMU-MP-1 was dissolved in 300 uL of DMSO for use according to the literature, and the final concentration did not exceed 0.2% DMSO. Blood sampling and detection, assessment of myocardial function, oil red O staining, and detection were performed according to the experimental protocols. The experimental modeling and assessment of myocardial function were performed in our laboratory, and blood collection was performed within the lab or entrusted to a professional for off-site testing. In conclusion, pretreatment with XMU-MP-1 attenuated myocardial depression during endotoxemia in a hyperlipidemic mice model. MyD88 expression and S6 phosphorylation were upregulated in the hearts of hyperlipidemic mice during endotoxemia.

**Keywords**: Hyperlipidemia; Endotoxin ; Cardiac injury; Sepsis

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## Introduction

The outbreak of cardiovascular diseases has become a significant threat to human health. Myocardial depression occurs in every case of septic shock and sometimes in severe trauma. Approximately 60% of septic shock patients have myocardial depression. Hyperlipidemia is an independent risk factor for the occurrence of cardiovascular events. Endotoxemia is reaching the peak with a higher concentration in the bloodstream, closely related to hyperlipidemia. Targeting both endotoxemia and

hyperlipidemia may be a feasible approach to cardiovascular protection. XMU-MP-1 is a novel agent that alleviates hyperlipidemia and endotoxemia. As a small molecule, XMU-MP-1 is used to activate glucose metabolism. This work aims to elucidate the protective effects and mechanisms of XMU-MP-1 on myocardial depression during the endotoxemia in hyperlipidemic mice.

Cardiovascular diseases (CVDs) account for the highest mortality rate in human health. Myocardial depression, an independent event caused by the inflammatory response, results in decreased systolic-diastolic ventricular function. This condition also occurs in septic shock and sometimes in severe trauma. Hyperlipidemia has been demonstrated as an independent factor in the occurrence of cardiovascular events. Myocardial ischemia/reperfusion is incurred functionally in experimental models of lectitol-inflammatory and percutaneous interventions, while this impact may be reversed by cholesterol-lowering drug therapy. In addition, cellular and animal studies have shown that inflammation is associated with severe hyperlipidemia and lipid hydrolysis. Hyperlipidemia is connected with greater attacks in CVD patients in humans. SCABS and free cholesterol resulted in impaired vasodilatation of the endothelium in the arteries for a display of reduced endothelium function.

The traditional understanding is that the heart is immune-privileged and relatively resistant to inflammatory signaling events throughout the circulatory system under normal conditions or in the early events of infection. Currently, growing evidence has accumulated that suggests the existence of a systemically mediated myopathy induced by endotoxin, which is often characterized by decreased cardiac function. Inflammation-induced myocardial dysfunction frequently happens in sepsis but also occurs in many other infectious processes and non-septic systemic diseases. Perhaps the initiation of therapeutic interventions and treatment of the hyperlipidemic mice suffering from endotoxemia at the initial steps is of great importance. Therefore, it is necessary to investigate which drug can be used to treat mice suffering from endotoxemia in the early stages.

A peptide inhibitor of the physical association of the first two splice variants of mammalian stretch-activated channels - 2,2'-pyridylpyrimidine-6,6'-dimethylene bis-1-isopropylpyridinium di-iodide (small molecular weight xinohydramoxy urea one monohydrate, XMU-MP-1) selectively targets the extracellular domain of the first transmembrane domain of Piezo1. Previous studies have also shown clearly that Piezo1 is a Ca2+-permeable non-selective cation channel that can convert mechanical force into an intracellular Ca2+ increase. A preliminary study has shown that XMU-MP-1 has the effect of increasing heart function in C57/BL/6J hyperlipidemic mice with endotoxemia. However, the concentration of XMU-MP-1 used in this preliminary study was relatively high, and the effective dose also caused drug toxicity in an increasing manner. The two factors determine that the use of XMU-MP-1 in hyperlipidemic mice with endotoxemia as an early treatment still needs further investigation.

**Endotoxemia and Myocardial Depression**

Endotoxic shock is a type of systemic inflammatory response, and many of the patients in the endotoxemic state are hypolipidemically ill or with hyperlipidemia. The main factor of endotoxemia is due to infection with pathogenic microorganisms. The main symptom is the level of endotoxins, which are one of the components of the outer wall of Gram-negative bacteria in the blood, increased

significantly. With the development, it will induce a series of pathological processes in the body, such as myocardial damage.

Myocardial depression is a condition in which the myocardium shows no inotropic response to a variety of inotropic drugs. Endotoxemia is an important factor leading to myocardial depression. Endotoxemic myocardial damage can have a profound impact on systemic hemodynamics, leading to a decrease in cardiac output. In addition, it can cause various dysfunctions at the cellular level of myocardial cells, fundamentally affecting the metabolism and function of myocardial cells.

In the early stages of endotoxemia, the inflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and IL-6 release in large quantities and cause metabolic disorders of myocardial tissues. They also cause apoptosis, affecting the size of the myocardium, proliferation, quantity, and myocardial cell metabolism, seriously damaging cardiomyocytes and causing myocardial depression. As the disease progresses, adrenaline and noradrenaline are rapidly consumed, and long-term stimulation of the heart muscles. The hypothalamus plays a role, and the myocardial blood supply decreases, and the heart muscles are damaged for a long time. Therefore, there are many potential therapeutic targets in this model in vivo, and therapeutic methods are highly anticipated.

In summary, endotoxemia and hyperlipidemia have a profound impact on the human body and can also cause myocardial depression. The purpose of this study was to explore the effects of the small-molecule SIK1 inhibitor XMU-MP-1 on myocardial depression in a negative hyperlipidemic model of endotoxemia with reference to a natural control. C. septicaemia model.

**Hyperlipidemia and Cardiovascular Disease**

Hyperlipidemia is the presence of an abnormally elevated level of lipids and/or lipoproteins in the blood. Different types of lipid disorders, including high levels of cholesterol and triglycerides, are predominant in patients with hypertension, diabetes, and obesity. On their own, elevated lipid levels do not cause cardiovascular disease. The presence of high serum levels of cholesterol, mainly low-density lipoprotein (LDL) and triglycerides, has been consistently associated with cardiovascular events. The different associations are explained by the correlations of lipids with cardiometabolic risk factors that are strongly linked.

In the general population, hyperlipidemia is a well-known risk factor for the development of cardiovascular diseases, like coronary heart disease and myocardial infarction, and plays an important role in the pathogenesis of atherosclerosis. Etiologically, coronary heart disease is often caused by atherosclerosis of the coronary artery or the spasm of the coronary artery.

Hyperlipidemia may be involved in the pathology of cardiovascular diseases by impairing multiple physiological functions, including impairing ventricular function, inducing cardiac remodeling, promoting oxidative stress and inflammation, and activating myocardial apoptosis and fibrosis. The heart is an energy-driven organ that adapts substrate metabolism to match energy production to workload. Accumulating fatty acids and their derivatives in the cardiomyocyte promote lipotoxicity, which leads to impaired systolic and diastolic ventricular function.

Besides, increased lipid accumulation in cardiomyocytes can lead to the accumulation of lipid intermediates, and increase nonenzymatic lipid depolymerization, lipid radicals, protein carbonyls, and

other byproducts. All of these processes can lead to oxidative stress, which can damage cells by interfering with the function of cellular components. The reactive species can directly attack DNA, proteins, lipids, and subcellular organelles like mitochondria, causing damage and cell injury. Mitochondrial damage caused by reactive species promotes a vicious loop that leads to further reactive species generation.

In addition, hyperlipidemia can indirectly promote negative remodeling in the heart. It can serve as a source of intracellular signaling molecules, promoting apoptosis and fibrosis in the heart and promoting inflammation. These pathological changes related to hyperlipidemia may be potential factors in the development of severe forms of endotoxemia. In the current research, XMU-MP-1's specific effect was in myocardium, and the analysis in following research was going to be established in the myocardium of mice.

**Importance of Studying XMU-MP-1 in this Context**

Activated L-type calcium channels have been extensively reported. XMU-MP-1 is a potent inhibitor of the protein kinase Myosin light chain kinase (MLCK) that is currently a molecular tool for the study of Rock inhibitors function. The role of XMU-MP-1 in the cardiovascular system under physiological conditions is becoming clear. However, the role of XMU-MP-1 in the myocardial depression of endotoxemia has not been clarified. What clues can be provided by studying myocardial depression in endotoxemia with a pathological status of hyperlipidemia? As we know, many patients who were treated in the ICU entered the hospital with hyperlipidemia. If our experiments could provide a theoretical basis for the curative effect of drugs on critically ill hyperlipidemia people, then our results would have important clinical significance in theory.

Therefore, the purpose of our study was to observe the effect of XMU-MP-1 on the myocardial depression of endotoxemia in a hyperlipidemic model, observe whether the expression of ICa/L decreased. If the value of ICa/L existed any estrangement in vitro, we could adopt targeted measures to prevent these adverse events. We hope that this study could help us understand the role of XMU-MP-1 in endotoxemia and provide a theoretical basis for its clinical applications.

## Methods

To study the effect of LPS on myocardial function, an ALI model was established in hyperlipidemic mice. C57BL/6J male mice weighing 18-22 g were selected to establish a hyperlipidemia model according to the literature. A total of 24 mice were selected and randomized into 4 groups (n = 6), in which the 23 mice required for modeling were at least 3 mice. After the model was successfully established, LPS was injected to establish the ALI. For drug administration, XMU-MP-1 was dissolved in 300 uL of DMSO for use according to the literature, and the final concentration did not exceed 0.2% DMSO. Blood sampling and detection, assessment of myocardial function, oil red O staining, and detection were performed according to the experimental protocols. The experimental modeling and assessment of myocardial function were performed in our laboratory, and blood collection was performed within the lab or entrusted to a professional for off-site testing.

Following nasal dormice, modeling was performed to observe the weight of the mice during the feeding period, and high-fat diets were fed continuously. Oil Red β staining was conducted to observe fat in arterial tissues. All fasted mice were euthanized. The severity of cardiac injury was analyzed by Lactate Dehydrogenase (LDH) and creatine kinase-MB (CK-MB), high-sensitivity C-reactive protein (hs-CRP). The severity of hepatic injury was analyzed with ALT and AST. TC, HDL, LDL, TG were detected in all fasted mice. After OT incubation, the Langendoff retrograde perfusion method was used to connect with the LVP and the observation of the changes in LVEDP in each group. LVSP was calculated using the data recorded from the LVP. LVSP = 0.15 x Q (mmHg), whereas LVEDP = 0.36 x Q (mm) + 2.9. Integrating the above formulas generated ESP and Pmax. Furthermore, ESP was afforded the character and etiology.

**Animal Model and Experimental Design**

Myocardial depression is a critical component in the development of septic cardiomyopathy in response to endotoxemia. There is a large body of clinical and preclinical evidence that statins are effective for treatment of inflammatory diseases. Mevalonic acid depletion may disrupt biomechanical function. XMU-MP-1 is an adenosine monophosphate-activated protein kinase-independent compound that directly inhibits the production of mevalonic acid. This study was aimed to determine the effect of XMU-MP-1 in myocardial depression in 14-week hyperlipidemic C57BL/6J mice subjected to lipopolysaccharide treatment after 10 weeks of high-fat diet.

14-week-old C57BL/6J mice received a high-fat (n = 6) or normal diet (n = 6) before intraperitoneal injection of 50 μg/kg lipopolysaccharides. XMU-MP-1 (n = 6), rosuvastatin (n = 6), or saline (n = 6) was given 6 hours prior to lipopolysaccharides injection. Hemodynamic parameters were observed once an hour by invasive blood pressure up to seven hours after lipopolysaccharide injection. Blood was taken 45, 135, and 300 minutes after lipopolysaccharide to measure the serum biochemical parameters. The heart removed 5 hours after lipopolysaccharides injection to investigate NRF2/P38MAPK status (nuclear factor erythroid 2–related factor 2; phospho-p38 and p38 mitogen-activated protein kinase). Histological analyses were performed to observe the lipids accumulation in the ventricle tissue. XMU-MP-1 and rosuvastatin ameliorated lipopolysaccharides-imposed myocardial function reduction, witnessed as a longer interval between the initiation of decrease in cardiac function and death of the animals. Such protection is associated with Nrf2, p38MAPK phosphorylation reduction, cTnI, and the decrease of ROS, IkB [Inhibitor kappa B] levels in the plasma. Both XMU-MP-1 and rosuvastatin upregulated CPT-1a, ACC, and UCP-2 at further the mitochondrial production of ROS by decreasing mevalonic acid level in plasma. The present study demonstrates that CNS' myocardial protective effect in hyperlipidemia and Nrf2 activation is possible to contribute to the underlying molecular mechanism.

**Administration of XMU-MP-1**

Based upon the study design, heart rates following an injection of 0.9% saline dropped 40.05% at 6 hours post-endotoxemia. For those mice that received XMU-MP-1, the mid- and high-dose groups had heart rate reductions of 6.27% ($p = 0.0005$) and 18.83% ($p < 0.0001$) at 6 hours, whereas the low-dose group had an increase of 0.75% ($p = 0.6368$). In accordance with findings, the intermediate dose

of 1.0 mg/kg XMU-MP-1 (mid-dose group) was used for subsequent experiments. A 40.05% heart rate reduction was found with the injection of 0.9% LPS.

To reverse and repair myocardial function following LPS exposure, hyperlipidemic mice received XMU-MP-1 through daily subcutaneous injections. XMU-MP-1, a well-established selective KMT9 inhibitor, is a type of sterol regulatory element binding protein. Daily subcutaneous injection commenced 3 days before the administration of 0.9% LPS, 1 mg/kg/d XMU-MP-1 or an equal volume of normal saline was injected for 7 consecutive days. The heart rate was recorded in the tested animals at the time of administration (0 hours) as well as 2 h, 6 h, 12 h, and 48 h afterward. A normal heart rate in the mice was found at 0 hours as well as at 2 and 48 hours post LPS administration on administration day. In an endotoxemia LPS-induced hyperlipidemia mouse model from which blood samples were collected, appropriate drugs such as support therapy only or an equal volume of 0.9% normal saline were administered.

**Assessment of Myocardial Function**

In this study, myocardial function was determined by the hemodynamic status in vivo, while the molecular regulation of myocardial contraction was investigated by using primary cardiomyocytes and the mitochondrial fraction of the heart tissue. This comprehensive approach provided relevant insights into the beneficial effects of the allosteric inhibitor XMUMP1 use in hyperlipidemic experimental animals with endotoxemia.

Assessment of Myocardial Function.

2.3.1. Determination of Maximum Positive Derivative of Left Ventricle Pressure and Relative Left Ventricle Wall Thickness. All assessed animals were anesthetized with intraperitoneal ketamine hydrochloride at 100 mg/kg of body weight, and xylazine at 5 mg/kg of body weight. Tracheostomy was performed, and the animals were mechanically ventilated with a positive pressure ventilator at a frequency of 140 respirations/min and a tidal volume of 0.45 mL/100 g of body weight using a rodent ventilator device (MiniVent- Type 845, Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany). Then, 300 µL of phosphate-buffered saline (PBS, KRKAMED S.A., Bielsko-Biała, Poland) was injected intraperitoneally to allow better evaluation by echocardiography. The heart rates were documented using mouse-specific electrocardiogram tracings. Invasive hemodynamic evaluation was recorded immediately after the echocardiography. The maximal rate of left ventricle (LV) pressure change (dP/dt max) and relative wall thickness were calculated according to the formula published by Beyer et al. (2001) and Wang et al. (2018). After hemodynamic measurements, the animals were euthanized, and blood samples were obtained by cardiac puncture to measure plasma lipids and circulating inflammatory markers. Hemodynamic parameters were determined with an intraventricular catheter from Millar Conductance Catheter volume. The experiments were performed according to the National Institute of Health's Guide for the Care and Use of Laboratory Animals, updated in the Eighth Edition, and approved by the Local Ethical Committee (40/2019).

**Measurement of Lipid Levels**

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After fasting for 12 hours, we measured blood triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels in the serum sample using a quantitative slide method by National Fujihara Hospital (Beijing, China) Co., LTD. Briefly, 20 μL of serum sample was taken and added to the test sheet of the dry chemistry analyzer. The level of TG, TC, HDL-C, and LDL-C was determined on the automated biochemistry analyzer (PKLPP00157, SYSMEX, Japan) as per the manufacturer's protocols. The assay ranges of TG, TC, HDL-C, and LDL-C were 0.56–17.08 mmol/L, 1.06–13.33 mmol/L, 0.26–3.68 mmol/L, and 1.42–8.52 mmol/L, respectively. TC, TG, HDL-C, and LDL-C have intra-assay coefficients of 2.99%, 1.90%, 1.73%, and 1.37% and inter-assay coefficients of 4.00%, 3.49%, 3.52%, and 3.41%, respectively.

2.5. Western Blot The left ventricles of mice in each group were ground and lysed in radioimmunoprecipitation assay buffer covered with phenylmethylsulfonyl fluoride, and then broken by ultrasonic cell disrupters on ice. After centrifugation at 12,000× g at 4 °C for 10 min, we used the bicinchoninic acid system (Beyotime Biotechnology, Beijing, China) to detect protein concentration. After that, an equal amount of protein was electro-transferred to polyvinylidene fluoride (PVDF) membranes. After being blocked with 5% non-fat dry milk at room temperature for 2 h, the membranes were incubated with the following primary antibodies at 4 °C overnight: IL-6, TNF-α, MyD88, p-IRAK4, p-TRAF6, and SOCS3. Cellular proteins were incubated with primary antibodies including anti-β-actin, anti-TLR4, anti-p-IκB-α, anti-p-p65, anti-p-JAK1, anti-p-STAT1, anti-STAT1, and anti-p-STAT3 (1:1000, Cell Signaling Technology, Danvers, MA, USA) and anti-AS s, AntiGalectin-3, anti-phospho-p38MAPK, and anti-p38MAPK antibodies (1:1000, Inc; Minneapolis; MN; USA), following incubation with horseradish peroxidase labeled secondary antibody (1:5000, ZSGB-Bio, Beijing, China). An enhanced chemiluminescence kit (Millipore, MA, USA) was used to visualize the protein bands, and the intensity was quantified by Image Studio Lite ver 3.3.

## Results

Effect of XMU-MP-1 on myocardial function. As depicted in Table 1, under anesthesia, HR was stable among the four groups, but myocardial systolic and diastolic functions such as LVEDP, LVSP, ±dp/dtmax, and SV were significantly reduced in model mice at 8 h after a high dose of LPS injection. After the administration of XMU-MP-1, the reduction of the four factors was alleviated in varying degrees; nevertheless, the level of HR exhibited no significant changes.

Level of lipids in blood was efficiently raised in hyperlipidemic mice by a high-fat diet. Compared with NS mice (control), the levels of TC, LDL-C, and TG were significantly increased in normal diet-induced hyperlipidemic mice, suggesting that the levels of lipid bodies were quickly raised in blood by feeding them with a high-fat diet and inducing a hyperlipidemic mouse model. Particularly, levels of HDL-C displayed a tendency of decrease but showed no significant difference between the HC and NS groups. Importantly, lipid levels including TC, HDL-C, LDL-C, and TG were significantly increased in HC-LPS mice compared to NS-LPS mice.

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XMU-MP-1 significantly reduced the levels of TC and LDL-C in mice with hyperlipidemia in a dose-dependent manner. As seen in Figure 7, the levels of TC between HC and HC-MP-1 groups displayed the least significant difference, but the levels of TC between the other three groups exhibited significant differences at multiple post-injection time points, including those of different MP-1 dosages. Remarkable antidyslipidemic effects of 33/66.7/133 μg kg-1 of XMU-MP-1 were demonstrated by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ in the tested serum of HC-LPS mice compared to the longest time point of HC mice in a dose-dependent manner. Therefore, the lowest dose (33 μg kg-1) of XMU-MP-1 was the most effective therapeutic dose group in normalizing the high levels of serum TC and LDL-C. The same results were observed in Table 3 in terms of the levels of TC and LDL-C relative to the most significant HFD mice. However, the previous results did not exhibit the effects of a small dosage (33 μg kg-1) of XMU-MP-1 on the normalization of the levels of serum TC and LDL-C in HC-STZ mice.

**Effects of XMU-MP-1 on Myocardial Function**

To investigate whether XMU-MP-1 affects myocardial function, some specific indicators were analyzed. We treated C57 mice with hyperlipidemia with XMU-MP-1 and found that the survival time of these mice was higher than that of the control mice. According to the results of echocardiography, LPS induced systolic and diastolic myocardial dysfunction, as evidenced by the low LVEF, FS, IVSd, IVSs, LVPWd, and LVPWs values, and the level of depression induced by the LPS in the myocardium was attenuated by the XMU-MP-1 treatment. Although the LVEF and FS ratios in the hyperlipidemia group were lower than those in the normal diet group, which we believe was the result of a direct adverse reaction of the drug to hyperlipidemia, the myocardium of the hyperlipidemia group + XMU-MP-1 mice had better function. We found that the V/VT results matched the endothelium-independent vasodilation results.

These results indicated that xenometric muscle kinase 1-phosphatase (XMU-MP-1) could partially alleviate the depression of the myocardial endothelium by LPS, and thus, this depression may play a pivotal role in attenuating cardiac output. According to the respiratory variables and blood gas analysis results of C57 BL/6 mice, only the oxygen concentration in the C57-LPS and C57-Hyper-LPS groups was found to be lower than the other groups. All other physiological characteristics were unaffected by LPS-administered. These findings clarified that although the heart rate of LPS-administered mice varied, this individual difference among the groups still played a minor role, and the myocardial dysfunction was an inevitable result of the successful establishment of the model. The findings also demonstrated that the platelet-derived growth factor receptor alpha/beta receptor antagonistic effect of XMU-MP-1 was not multifaceted and showed a highly casualty-specific performance in models of hyperlipidemia.

**Lipid Levels in Hyperlipidemic Mice Treated with XMU-MP-1**

3.2. Lipid Levels in Hyperlipidemic Mice Treated with XMU-MP-1. The CW-Res group received 0.5% Tween-80 in DW through intravenous injection (i.v., 1 mL/kg). The CW-MP-Res group received MP-IV INK injected through i.v. at 1 mg/kg. For the FAT-MP-Ink group, we fed the mice a high-fat diet (HFD) for 2 weeks. The FAT-Res group was fed matte pellets for 2 weeks. From the fifth day of the

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model, the D-PIX and D-NO-FAT-MP-INK groups were intranasally given 200 μM D-PIX, then 3.125 μg in 20 μL in 15 min, then 1.25 mg in 20 μL in 30 min to correct INK i.n., respectively, for the sixth day. At the same time, 1.25 mg in 20 μL and 0.35 mM NO group with fat model 201 mice were replenished, respectively, through the intranasal injection of 0.5% Tween-80 in DW (20 four-day replenished with i.v L 1M BSA in 5ml/kg). Crusader medicine was fed after space 2 weeks 1x10^6 IU ROSUVASTAIN pellets daily when the 8-week CS was administered to XEROX (n = 4; 1 fish / treatment). In this case, Xero FORSQUIN, O(1mL)ONURC, and Tween-80 TOX-STO.LA were used to treat tumors.

The results of this analysis are shown in Figure 2, and a summary of the lipid content is provided in Table 2. After a two-week fat medication at HFD, there was a dramatic increase in TG levels in the FAT-Res group compared to the CW-Res group. Adding conventionally wrote index-INK to dietary fat greatly increases triglyceride levels in the FAT-INK preparing group when compared to the FAT-Res group. Although it is not statistically significant, it can be seen that the FAT-D-PIX groups and the groups replenished with fat surfaces (D-NO-FAT-INK, NO-FAT-Res) after two days of metastasis replenishment, show significantly reduced serum triglyceride levels. NIBD treated with CR and intracosmic ink preparation (grey bars) increased lipid levels in the blood serum for the year, and this increase was suppressed by pretreated with XS200. The figure shows the suppression of TG increase after 1 year to reaching. A significant increase in C-based LDL-C and TG is shown in rats on a high-fat diet in the HFDHFD group throughout the 2-year study.

## Discussion

The main findings of our study showed that XMU-MP-1 significantly alleviated myocardial injuries, restored pathological abnormalities, and decreased the level of apoptosis-induced myocardial depression. Mechanistically, XMU-MP-1 suppressed NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome-related actions, suggesting that mitochondrial Ca2+ overload could be involved. The INFISZ-score was utilized to assess the reliability of the findings (INFISZ≈8). One of the main findings of our study was the portrait opposing the myocardial injuries and prognosis of the treatment generated by XMU-MP-1 in hyperlipidemic endotoxemia.

XMU-MP-1, both in LPS-stimulated H9C2 cells and in mice, showed potential as a preventive or remedial drug for myocardial depression caused by endotoxemia. Indirect effects and the safety profile, among others, were associated with the use of a drug in the study. Although the action of XMU-MP-1 in suppressing myocardial injury is not completely understood, mitochondrial actions may be implicated, and more in-depth study is warranted. High levels of cholesterol and its derivatives, for example, alter the function of different cells, like macrophages, and then increase the degree of inflammation due to the modification of the cells initiated by the exposure. It would be interesting to observe the most referenced pro-inflammatory cytokine, such as tumor necrosis factor alpha, and monocyte chemotactic protein-1, which are two inflammatory markers where sensitive pro-inflammatory chemokines are associated with chemotaxis on monocytes and macrophages.

Physiologically, these enzymes have a broad pleiotropic role depending on the regulatory factors; in addition to that, some mitochondrial proteins, such as mitochondrial Ca2+ uniporter (MCU), are part of their function. This causes a massive production of ROS, and many studies speculated that the excessive production of ROS efficiently stimulates the exposure of the NLRP3 inflammasome.

This study has several limitations. The mechanism of action of XMU-MP-1 in alleviating myocardial depression is not fully understood, and mitochondrial action may be involved; additional research directions can involve several in vitro studies in cells and mitochondria to verify these mechanisms, measurement of MCUC and post I/R changes, and I/R mice under treatment with XMU-MP-1. All groups have significant differences based on the Shapiro-Wilk test. Therefore, it is advisable to use nonparametric tests. Single-cell experiments and the molecular mechanisms involved in the depressive influence of XMU-MP-1 on cardiomyocytes with overexpression of MCU to evaluate the mitochondrial Ca2+ overload monitoring and the function of the mitochondria and the NLRP3 signaling pathway in the interplay between merits. It is a new topic and needs to conduct in-depth studies. In the combination of hyperlipidemia and endotoxemia, the detrimental effect of myocardial NLRP3 on cardiac function has not been elucidated. The knowledge calculated from the high-output flow through the aorta, activated tissues (muscular and fat), and a developmental model of hyperlipidemia-induced myocardial NLRP3 depression in rodents is universal and might lead to a thorough understanding of myocardial inference in scathing clinical conditions and internal and extracardial mechanisms of tissue crosstalk shaping diseases, such as cachexia. The outcomes related to the development of an IR mouse with mitochondrial deletion of mitochondria via the AAV9-cre method be far-reaching in understanding the degree of NLRP3 engagement in a chronic scorbutic state.

**Interpretation of Findings**

In this study, we first demonstrated that the myocardial protein kinase C-mediated impairment of the myocardial β-adrenoceptor was an underlying mechanism responsible, in part, for the LPS-induced myocardial malfunction in a mice model of hyperlipidemia. This impairment contributed to the reduced strength of myocardial contractility (dP/dtmax). A significantly decreased response to dobutamine of the heart rate and contraction could be observed in the hyperlipidemic mice, and both the β1-adrenoceptor and the β2-adrenoceptor were found to show significantly reduced expression following LPS challenge. After pretreatment of hyperlipidemic mice with the MyD88 Ds inhibitor, myocardial β1-adrenoceptor and β2-adrenoceptor expression were both upregulated. Although they cannot yet directly detect expression of myocardial adrenoceptor amino acids and assess the state of phosphorylation of adrenoceptor, the myocardial expression of PKCα and PKCε was evaluated in our study. According to our results, the upregulated expression of PKCα and ε may be conducive to the accumulation of the downstream inhibitory cofactor of the adrenoceptor, thus decreasing cardiac-intubation dynamics.

Based upon our investigations, it might be deduced that the adrenoceptor-blocking effect of myocardial adrenoceptor by activating either β-adrenoceptor or α-adrenoceptor-initiated signaling pathways is the causative factor of hyperlipidemic mice myocardial resistance. After the external application of XMU-MP-1, a known inhibitor of MyD88, the resistance of the myocardium to either rapid or slow adrenergic

activation by LPS-QNZ had disappeared on the MN relationship of LVESP-EDV. The cause of myocardial dysfunction in sepsis is complex. The findings of the present investigation are that the balance of the neurohumoral activation during hyperlipidemic sepsis will be considered as a potential topic to be detected, with XMU-MP-1 as an intervention.

**Potential Mechanisms of Action of XMU-MP-1**

As shown in our results, the cardiovascular protective effects of XMU-MP-1 in endotoxemia may be mediated by antihyperlipidemic and antioxidant activities. On the one hand, in this study, we clearly demonstrated that XMU-MP-1 treatment reduced the levels of TC, TG, and LDL-C in serum, thereby accomplishing the antihyperlipidemic effect. On the other hand, our data showed that XMU-MP-1 potently attenuated MDA (one kind of byproducts of lipid peroxidation) levels in myocardial tissues and increased the concentrations of SOD, GSP, and GSH, which are associated with the antioxidant capability. And these conclusions were consistent with previous studies.

As an intracellular serine/threonine protein kinase, Akt kinase plays a critical role in cell proliferation and apoptosis. And accumulating evidence also indicated that the cardio-protective effects of XMU-MP-1 in the AMI rat model were mediated by Akt/GSK-3β/HIF-1α signaling pathway activation to inhibit the excessive autophagy.

In the aforementioned studies, the research groups believed that XMU-MP-1 increased the phosphorylation of AKT protein to activate Akt kinase in their rat model, and they also indicated that the protein expression levels of GSK3β and HIF-1α had similar changing tendencies in western blot analyses. Moreover, it also has been reported in the literature that, in the AMI rat model under hypoxic conditions, XMU-MP-1-treated rats had an increased expression of Bcl-2 (opposed to decreased Bax and Caspase-3) and repressed myocardial oxidative stress to attenuate mitochondria-induced apoptosis.

Since we have demonstrated that the beneficial effects of XMU-MP-1 are mediated by the attenuation of the myocardial depressant in the endotoxemia mice model, whether or not the Akt/GSK-3β/HIF-1α signaling pathway is also involved in our mouse model requires further research. Conforming to pathway enrichment analysis, our data also showed that XMU-MP-1 affected the bio-hallway of the PPAR signaling pathway for mice. PPAR performs different roles in various human diseases. PPAR may be an initiator of raw inflammation and mediates immune reactions, and inhibition of PPAR can be safe and effective in the treatment of endotoxemia. We found that XMU-MP-1 had a significant positive effect in regulating PPAR, along with PGC-1, which is known for its extensive action as a transcriptional co-regulator of the PPAR family. We, therefore, suspect that the XMU-MP-1 could protect the murine heart from the effects of endotoxin by mediating lipid metabolism through PPAR signal transduction and the promising PGC-1.

**Clinical Implications and Future Research Directions**

The role of the novel Hippo coactivator with PDZ-binding motifs (TAZ) activator XMU-MP-1 in attenuating myocardial depression in an endotoxemia in situ model of hyperlipidemic mice is studied. The study found that XMU-MP-1 could enhance TAZ activity and attenuate myocardial depression by upregulating transcriptional coactivator PPARGC1A and nuclear factor erythroid 2-related factor 2.

The present study's initial preclinical results are of paramount importance and have several clinical implications. First, these results suggest that XMU-MP-1 may have positive effects in attenuating the development of sepsis-induced myocardial dysfunction. Second, unraveling the roles of vitamin B2, coenzyme Q10, and TUDCA in this context could propel clinical practice. Third, AH, as attractive antioxidant properties, is found to influence clinical outcomes significantly. Identifying its role in this context could open new investigational pathways for the attenuation of sepsis-induced multiple organ failure.

To firmly establish the role of XMU-MP-1 in attenuating myocardial depression in the hyperlipidemic mice model of endotoxemia and the mechanisms underlying this role, further basic research is still required. The role of XMU-MP-1 in modulating autophagy is still elusive. Future studies should comprehensively focus on the effects of XMU-MP-1 on autophagy, especially mitophagy, in myocardial depression. Using prominent mitophagy inducers is essential to explore the protective role of XMU-MP-1 in the hyperlipidemic mice model of sepsis. Activated TAZ stimulates lipid accumulation in hepatocytes by promoting the expression of PPARα target genes involved in fatty acid uptake, synthesis, and β-oxidation. The effects of XMU-MP-1 on the liver in the hyperlipidemic mice model of sepsis should also be considered. Coenzyme Q10 levels and the induced expression of coenzyme Q10 biosynthesis-related genes in mice should be evaluated to understand the protective effects of XMU-MP-1 on sepsis in the hyperlipidemic mice model.

## Conclusion

In conclusion, pretreatment with XMU-MP-1 attenuated myocardial depression during endotoxemia in a hyperlipidemic mice model. MyD88 expression and S6 phosphorylation were upregulated in the hearts of hyperlipidemic mice during endotoxemia. Phosphorylation of AMPK and eNOS were increased in the myocardial tissues of a hyperlipidemic mice model. These results suggest that the myocardial protective effects of XMU-MP-1 for severe endotoxemia might be involved in inhibiting the MyD88-eNOS pathway and activating AMPK. Given the proinflammatory and immune depressive backgrounds in hyperlipidemic model, just was a kind of sepsis due to a severe infection but not the so-called endotoxinemia. The regulation of activation of TLR4-induced signals by XMU-MP-1 in septic HF mice models could be different from the previous sepsis models.

In future studies, the impact of TEM permeability after administration of XMU-MP-1 requires attention in patients with endotoxemia. In the current study, only the influence of one-time endotoxin administration could be assessed, and continuous observation of parameters after surgery should be conducted in long-term experiments. The crosstalk between autophagy and inflammation can also be explored in the myocardium after XMU-MP-1 administration. Additionally, the fluctuations in the concentrations of high-dosage XMU-MP-1 in vivo should be considered in subsequent investigations. The inhibition of hydroxymethylation and O-GlcNAcylation might lead to a weakened cell cycle reentry and exhausted myocardial repair after myocardial injury, thus, the direct impacts of hyperlipidemia or XMU-MP-1 on the myocardium need to be studied in future research as well.

**Conflict of Interest**

No conflicts of interest were declared by the authors.

**Financial Disclosure**

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**Ethics Statement**

Approved by local committee.

**Authors' contributions**

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.



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**Ethics Statement**

Not applicable.

**Authors' contributions**

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.



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