Cannabidiol Attenuates Methamphetamine-Induced Cardiac Inflammatory Response and Necrosis through The PKA/CREB Signaling Pathway

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Abstract

Methamphetamine (MA) abuse is a major global public health problem, with cardiovascular issues becoming an increasingly recognized complication. Cannabidiol (CBD) has gained recent attention, due to its various pharmacological properties. However, whether CBD has therapeutic effects on MA-induced cardiotoxicity remains unknown. In the present study, we investigated whether CBD has a protective or therapeutic effect on MA-induced cardiac damage in rats via the protein kinase A (PKA)/cyclic adenosine monophosphate response element-binding (CREB) signaling pathway. Thirty rats were randomly divided into five groups. The rats were administered MA by intraperitoneal injection (IP) once a day for 4 weeks, with CBD (40 or 80 mg/kg, IP) treatment 1 h prior to the MA injections. Body and heart weights were measured, and morphological changes were determined using hematoxylin & eosin and Masson's trichrome staining. The serum levels of interleukin-6 (IL-6) and IL-10 were detected using enzyme linked immunosorbent assay (ELISA) kits. The protein expression levels of PKA, phospho-PKA (p-PKA), CREB, phospho-CREB (p-CREB) and cardiac troponin I (cTnI) in the myocardium were detected by western blot analysis. Results showed that the heart-to-body weight ratio increased significantly following MA administration but decreased with CBD treatment. Chronic administration of MA resulted in a cardiac inflammatory response and progressive development of fibrosis, while CBD treatment attenuated these lesions in a dose-dependent manner. MA administration increased IL-6 but decreased IL-10 levels, which were reversed by CBD pretreatment. Moreover, MA significantly increased the cTnI level, but this was decreased by CBD treatment at 80 mg/kg. The protein expression levels of PKA, p-PKA, CREB, and p-CREB increased following MA administration, but significantly decreased with CBD treatment. Overall, these results indicate that chronic MA administration leads to cardiotoxicity, including cardiac inflammatory response, fibrosis, and myocardial necrosis, but these effects can be attenuated by CBD pretreatment. Our research suggests a potential application of CBD for MA-induced cardiotoxicity, which may attenuate inflammatory response and necrosis through the PKA/CREB signaling pathway.

Introduction

Methamphetamine (MA) is a highly addictive class of synthetic stimulant and one of the most popular drugs of abuse worldwide(*World Drug Report 2020*). The number of young MA users has increased dramatically in recent years, resulting in criminal detention and medical resource shortages. Thus, MA abuse has become of increasing concern and is a major global public health problem(Kohno et al., 2020, Spivak et al., 2020). The molecular structure of MA consists of a methyl group added to the base structure of amphetamine (Fig. 1A). MA is highly lipophilic, allowing it to readily cross the blood-brain barrier(Homer et al., 2008), where it can affect the central nervous system by increasing catecholamine (e.g., dopamine and norepinephrine) expression via multiple mechanisms(Sulzer et al., 2005, Krasnova and Cadet, 2009, Reiner et al., 2009, Shaerzadeh et al., 2018). Many studies have focused on the neurotoxic effects, neuropsychiatric deficits, and addiction profiles of MA(Moratalla et al., 2017, Der-Ghazarian et al., 2019, Pan et al., 2020, Razavi et al., 2020). However, MA is a sympathomimetic amine substance, and can have a series of sides effects on multiple organs, such as the brain, heart, kidney, and

spleen, following overdose and chronic administration(Rusyniak, 2011, Gurel, 2016, Wu et al., 2016, Isoardi et al., 2020). Of note, cardiovascular injury caused by MA use has gained recent attention.

Hypertensive heart disease, pulmonary hypertension, delayed cardiomyopathy, and cardiogenic shock are frequently found in MA abusers, with sudden unexpected death also reported(Nishida et al., 2003, Segawa et al., 2019, Dalal et al., 2020, Hendrickson and Strauss, 2020). A retrospective study on autopsy found that cardiovascular disease is the second leading cause of death in MA abusers, after accidental drug toxicity(Darke et al., 2017). Furthermore, 68% of MA poisoning-related deaths are reported to show changes in cardiovascular pathology(Akhgari et al., 2017). Cardiovascular complications from MA are increasingly recognized and include vasoconstriction, hypertension, arrhythmia, aortic dissection, acute coronary syndromes, pulmonary arterial hypertension, atherosclerotic coronary artery disease, cardiomyopathy, and heart failure(Paratz et al., 2016, Kevil et al., 2019, Nishimura et al., 2019). The molecular mechanisms underlying MA-associated cardiovascular injury are likely multifactorial, intracellular calcium and potassium homeostasis defects, reactive oxygen species (ROS) generation, oxidative stress, mitochondrial dysfunction, and apoptosis may be related to direct damage(Liang et al., 2010, Lord et al., 2010, Reddy et al., 2020). MA can exert toxic effects indirectly via catecholamines due to its high affinity with dopamine and norepinephrine transporters(Han and Gu, 2006). Moreover, trace amino acid receptor 1 and sigma-1 receptor also play important roles via interactions with MA in the cardiovascular system(Nguyen et al., 2005, Lewin et al., 2011). High catecholamine levels can lead to vasospasm, hypertension, tachycardia, and myocardial ischemia(Won et al., 2013). Through the above pathophysiological mechanisms, MA eventually causes cardiac systolic dysfunction and inflammation, progressing to fibrosis and cardiomyopathy(Reddy et al., 2020).

Inflammation is an important risk factor for cardiovascular disease(Sethwala et al., 2021), and also plays a key role in MA-induced cardiac injury. Endomyocardial biopsies from the left ventricles of patients with MA-associated cardiomyopathy show increases in myocyte damage and markers of inflammation and fibrosis(Schurer et al., 2017). Myocarditis and endocarditis are also found in MA abusers during postmortem examination(Darke et al., 2017). The pathological development of cardiac inflammation can lead to arteriosclerosis, coronary heart disease, heart structure remodeling, and dilated cardiomyopathy(Gao et al., 2015, Kevil et al., 2019, Reddy et al., 2020). However, the molecular mechanism of myocardial inflammation induced by MA exposure has not yet been fully elucidated. Reducing mitochondrial membrane potential and promoting ROS expression induced by MA may potentiate the inflammatory process(Potula et al., 2010). Toll-like receptor 4 (TLR4) plays an important role in immune and inflammatory responses by activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which further regulates the expression of cytokines. The TLR4/NF-κB signaling pathway is involved in the pathology of MA-induced inflammation in BV2 cells, increasing the level of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β)(Wan et al., 2017). The cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA)/cAMP response element-binding (CREB) signal transduction pathway is involved in a variety of physiological and pathological processes, which can modulate working memory, cognitive function, fibrosis, and inflammation(Vandesquille et al., 2013, Li et al., 2015, Wang et al., 2015, Tanaka et al., 2020). However,

whether the cAMP/PKA/CREB signaling pathway is involved in MA-induced myocardial inflammation remains poorly understood.

Although autopsy, clinical, and animal studies provide compelling evidence of cardiovascular dysfunction, the Food and Drug Administration has not approved any specific treatments or drugs for cardiac complications caused by MA. Cannabidiol (CBD) (Fig. 1B) is a non-psychoactive component of *Cannabis sativa*(Mechoulam and Hanus, 2002), which is considered safe and well tolerated in high doses and chronic use(Bergamaschi et al., 2011, Shayesteh et al., 2019). In recent years, CBD has been applied in the treatment of various diseases, such as epilepsy, schizophrenia, addiction, anxiety, and neonatal ischemic encephalopathy(Devinsky et al., 2014, Sands et al., 2019), and shows multiple effects, including immunosuppression, anti-inflammation, anti-fibrosis, neuroprotection, and anti-oxidation activity(Garcia-Arencibia et al., 2007, Campos et al., 2017, Nichols and Kaplan, 2020, Sunda and Arowolo, 2020). Furthermore, CBD shows therapeutic effects on MA-induced reward and protective effects on impairment of recognition memory(Razavi et al., 2020, Yang et al., 2020a). However, whether CBD has a therapeutic effect on MA-induced cardiac inflammation remains unclear.

In the present study, we explored the potential protective effects of CBD and involvement of the PKA/CREB signaling pathway in MA-induced cardiac inflammation. We evaluated the therapeutic effects of CBD on cardiac inflammation induced by MA and the expression of key factors of the PKA/CREB signal pathway in the myocardium of rats, which may provide a novel strategy for the treatment of cardiotoxicity induced by MA abuse.

Materials And Methods

Reagents and chemicals

We legally obtained MA (purity above 98%) from the Yunnan Provincial Public Security Department. The MA was dissolved in saline and administered at a dose of 10 mg/kg via intraperitoneal injection (IP). The dose of MA was based on previous studies, with slight adjustment(Friend et al., 2013, Meng et al., 2020). The CBD was purchased from Hebei Fan Zhang Tang Commercial and Animal Husbandry Co., Ltd. (Cat. #: 13956-29-1, Hebei, China). It was dissolved in a vehicle solution of 5% dimethyl sulfoxide (DMSO) and 5% polysorbate 80 (Tween-80) in saline and then IP administered at doses of 40 and 80 mg/kg. The CBD dosages were selected according to the results of our previous study(Yang et al., 2020a). Tween-80 was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Cat. #: T8360, Beijing, China), and DMSO was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals and experimental design

Thirty male Sprague-Dawley rats (6 weeks old, 180–220 g) were purchased from the Laboratory Animal Center of Kunming Medical University (KMU). All animal studies were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of KMU, and all experimental procedures were approved by the Ethics Committee on Animal Care and Use from KMU (Approval code: kmmu2020403).

Rats were housed together (6 rats/cage) and provided with free access to food and water under standard conditions (room temperature of 22 ± 1 °C, humidity of 50%–60%, and 12-h:12-h light: dark cycle). All rats were acclimatized to the new environment for 3 days prior to the start of the experiments. The rats were randomly divided into five experimental groups (n = 6/group): i.e., solvent control group (saline solution containing 5% DMSO + 5% Tween-80, 10 ml/kg, IP), CBD_{80} group (CBD, 80 mg/kg, IP), MA group (MA, 10 mg/kg, IP), CBD_{40} (40 mg/kg) + MA group, and CBD_{80} (80 mg/kg) + MA group. The rats received MA or CBD once a day (at 09:00 am) for 4 weeks, with the MA (10 mg/kg) injections given 1 h after CBD treatment (40 or 80 mg/kg, IP), and weighed weekly for dose adjustment. All rats were sacrificed by narcotic overdose 24 h after the last injection. Cardiac blood (for serum) and hearts were collected. Some hearts were fixed in 4% paraformaldehyde solution, and some hearts were stored at −80 °C until further analysis. The experimental protocols are shown in Fig. 1C.

Histopathological examination

The hearts were fixed in 4% paraformaldehyde solution, dehydrated, embedded in paraffin wax, and sliced into 5-μm thick sections using a microtome (RM2235, Leica, Germany). The sections were then stained with hematoxylin & eosin (H&E) to assess inflammatory and necrotic changes in tissue and with Masson's trichrome to assess fibrotic changes. The heart tissue sections were examined using a digital pathological section scanning system (KF-PRO-005-EX, KFBIO, Ningbo, China)(Song et al., 2020).

Enzyme linked immunosorbent assay (ELISA)

The cardiac blood serum levels of pro-inflammatory cytokine IL-6 and anti-inflammatory cytokine IL-10 were measured using rat ELISA kits (Mlbio, Shanghai, China) in accordance with the manufacturer's protocols. Absorbance was recorded at 450 nm.

Western blot analysis

The left ventricles of the hearts were dissected and lysed in enhanced radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) containing 1% protease inhibitor and 1% phosphatase inhibitor on ice for 30 min, then centrifuged at 12 000 rpm for 15 min at 4 °C. Protein concentrations were measured using an enhanced bicinchoninic acid (BCA) protein assay kit (Beyotime, Shanghai, China). Equal amounts of protein (50 μg) were separated via 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred with a constant current onto 0.45-μm polyvinylidene fluoride (PVDF) membranes (Millipore, MA, USA). The membranes were sealed with non-fat dry milk (diluted in TBST buffer) for 2 h at room temperature, then incubated overnight at 4 °C with primary antibodies: rabbit anti-PKA (Proteintech, IL, USA, 1:1 000), rabbit anti-p-PKA (Abcam, Cambridge, UK, 1:1 000), rabbit anti-CREB (Proteintech, IL, USA, 1:1 000), rabbit anti-p-CREB (CST, Massachusetts, USA, 1:1 000), rabbit anti-cardiac troponin I (cTnI) (Proteintech, IL, USA, 1:2 000), and rabbit anti-GAPDH (Servicebio, Wuhan, China, 1:2 000). The membranes were washed with TBST three times, incubated with horseradish peroxidase (HRP)-linked anti-rabbit IgG secondary antibody (Abbkine, Wuhan, China, 1:5 000) for 1.5 h at room temperature, then washed again (as above). The membranes were detected using an ultra-high

sensitivity electrochemiluminescence kit (Biosharp, Beijing, China) and images were captured by the Bio-Rad imaging system (Bio-Rad, Hercules, California, USA). Protein bands were determined by Image J software and the intensities of each band were normalized to GAPDH. The experiment was repeated in triplicate and representative western blot images were presented.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). Statistical analyses were performed with SPSS v21.0 (IBM SPSS, Chicago, USA), and graphs were constructed using GraphPad Prism v6.0 (GraphPad Software, USA). One-way analysis of variance (ANOVA) was used to analyze experimental data, followed by Tukey's post-hoc tests for comparing individual groups. Paired *t*-test was applied to evaluate body weight between pre- and post-drug administration. Significance was defined at $P < 0.05$.

Results

Effects of CBD on MA-induced body and heart weight changes in rats

Body weight was measured before drug injection and at 1 to 4 weeks after drug administration. Body weight showed a time-dependent increase and was significantly higher after 4 weeks of administration than before administration in each group ($P < 0.01$, Fig. 2A). Weight gain was lower in the MA group than in the control group but showed no significant difference between the two groups after 4 weeks of administration. Compared with the MA group, CBD had no effect on body weight at either dose (40 or 80 mg/kg) (Fig. 2A). The hearts were weighed immediately after dissection. As shown in Fig. 2B, although heart weight increased slightly in the MA group compared to the control group, this increase was not statistically significant. Interestingly, however, 1-h pretreatment with 80 mg/kg of CBD reduced MA-induced cardiomegaly compared to the MA group ($P < 0.01$). The heart-to-body weight ratio increased significantly in the MA group compared to the control group ($P < 0.01$, Fig. 2C), whereas CBD (80 mg/kg) pretreatment for 1 h reduced the ratio compared to the MA group ($P < 0.01$, Fig. 2C). These results suggest that chronic exposure to MA for 4 weeks can induce cardiomegaly in rats, to some extent, which can be inhibited by administration of CBD (80 mg/kg).

CBD attenuates MA-induced cardiac inflammatory response

The H&E-stained heart tissues in the MA group showed myocardial fiber disorder, mild interstitial oedema, mild myocyte hypertrophy, vacuolization and karyolysis, as well as distinct mononuclear inflammatory infiltration in interstitial spaces or around blood vessels, and focal lesions with necrosis. As shown in Fig. 3C, focal mononuclear inflammatory infiltration was observed with myocardial necrosis and fibrosis. The myocardial structure of the control and CBD_{80} groups was mostly normal (Fig. 3A and 3B). However, pretreatment with 40 or 80 mg/kg CBD for 1 h before MA administration resulted in gradual and effective suppression of cardiac lesions. As shown in Fig. 3D and 3E, only slight mononuclear inflammatory infiltration in the interstitial spaces or around blood vessels was observed, mainly manifested as myocardial cell degeneration. Myocardial fibrosis is a progressive pathological process of chronic

inflammation. Here, Masson's trichrome staining was used to evaluate fibrosis in the myocardium, resulting in blue-brown-stained nuclei, red-stained cytoplasm and myofiber, and blue-stained collagen fibers. As shown in Fig. 3H, more blue-stained collagen fibrils in the perivascular and interstitial spaces were observed in the MA group than in the control group. Compared with the MA group, 40 mg/kg CBD pretreatment reduced the extent of fibrosis, although perivascular fibrosis remained (Fig. 3I), whereas there was almost no fibrosis in the 80 mg/kg CBD group (Fig. 3J). To further assess MA-induced inflammation and effects of CBD on inflammation, we evaluated the cardiac blood serum levels of pro-inflammatory cytokine IL-6 and anti-inflammatory cytokine IL-10 via ELISA. As shown in Fig. 4A, the level of IL-6 increased significantly in the MA group compared to that in the control group. However, IL-6 expression decreased dose-dependently following CBD pretreatment. In addition, MA significantly reduced the expression of IL-10 compared to that in the control group, whereas CBD pretreatment increased the level of IL-10 in a dose-dependent manner (Fig. 4B). Thus, chronic administration of MA in rats can cause a cardiac inflammatory response and progressive development of fibrosis, while CBD treatment shows protective effects against this pathology.

CBD attenuates MA-induced myocardial necrosis in left ventricle

To assess the extent of myocardial damage caused by MA exposure, we evaluated the left ventricle level of cTnI, an important biomarker of myocardial necrosis, via western blot analysis. As shown in Fig. 4C, MA significantly increased the expression of cTnI compared to that in the control group ($P < 0.01$), whereas pretreatment with 80 mg/kg CBD attenuated the level of cTnI compared to that in the MA group ($P < 0.05$), although no effect was observed under 40 mg/kg CBD.

CBD down-regulates PKA, p-PKA, CREB, and p-CREB expression in left

ventricle following MA-inducedcardiotoxicity

As shown in Fig. 5A and 5B, the expression levels of PKA ($P < 0.01$) and p-PKA ($P < 0.05$) were significantly higher in the MA group compared to the control group. However, pretreatment with 40 mg/kg or 80 mg/kg CBD reduced the expression levels of PKA and p-PKA compared to that in the MA group, with 80 mg/kg CBD more effective (PKA: $P < 0.01$; p-PKA: $P < 0.001$). As shown in Fig. 5C and 5D, the expression levels of CREB ($P < 0.01$) and p-CREB ($P < 0.05$) were significantly higher in the MA group compared to that in the control group. However, pretreatment with 40 mg/kg or 80 mg/kg CBD reduced the expression levels of CREB and p-CREB compared to that in the MA group. Interestingly, compared with 40 mg/kg CBD, 80 mg/kg CBD treatment did not significantly reduce the level of CREB, but was more effective for p-CREB ($P < 0.01$).

Discussion

Previous studies have indicated that body mass index and heart weight are significantly higher in MA abusers than in non-MA users(Abdullah et al., 2020). In addition, compared with deaths caused by multiple drug toxicities, those from MA toxicity show heavier hearts(Darke et al., 2018). In the current

study, we demonstrated that rat body weight increased significantly after 4 weeks of MA administration (Fig. 2A). These results are similar to previous findings showing an increasing trend in body weight in mice receiving MA (2 mg/kg, IP, 10 days), followed by withdrawal (7 days)(Garcia-Carmona et al., 2018), but differ from other research showing no significant differences in body weight in mice receiving MA (0–6 mg/kg) via subcutaneous injection 5 days a week for 4 weeks(Abdullah et al., 2020). Heart weight was higher in the MA group than in the control group, but not significantly. Relative heart weight, calculated as the heart-to-body weight ratio, increased significantly in the MA group compared to the control group (Fig. 2C), consistent with that found in mice (i.e., increased 4–5 mg/week for 8 weeks at 35 mg/kg MA and 2 mg/week after 20 weeks at 40 mg/kg)(Marcinko et al., 2019). In contrast, other research has reported no significant differences in relative heart weight in rats after an acute dose of 50 mg/kg MA or in mice after 10 days of exposure to 2 mg/kg MA(Islam et al., 2009, Garcia-Carmona et al., 2018). These discrepancies could be explained by the different concentrations and treatment times of MA administration, animal species tested, or specific experimental protocols used. The current study is also the first to report on the protective effects of CBD (80 mg/kg) against MA-induced cardiomegaly in rats.

Various autopsy reports have revealed that cardiomyopathy, coronary artery stenosis, valvular heart disease, and inflammatory heart disease are involved in many MA-toxicity deaths, with myocyte hypertrophy, myocarditis, endocarditis, pericarditis, perivascular and interstitial fibrosis, fiber necrosis, collagen deposition, and subendocardial myocardial infarction found via microscopic examination(Nishida et al., 2003, Darke et al., 2017, Darke et al., 2018, Abdullah et al., 2020). The non-specific cardiac histopathology observed in our study following MA exposure is similar with that reported in autopsy studies and animal models(Islam et al., 2009, Marcinko et al., 2019, Abdullah et al., 2020). Notably, inflammatory response, fibrosis, and necrosis of myocardial tissue were confirmed by H&E staining, ELISA analysis, Masson's trichrome staining, and western blotting. Compared to the MA group, however, CBD pretreatment alleviated these lesions to a certain extent.

Long-term MA administration in ApoE$^{-/-}$ mice can lead to a significant increase in the levels of plasma C-reactive protein, inflammatory cytokines (ICAM-1, VCAM-1, TNF-α), and neuropeptide Y in the aortic root and myocardial tissue, which promote inflammation and atherosclerosis(Gao et al., 2015). Furthermore, acute exposure to MA in mice (30 mg/kg for 6 h) results in a significant increase in serum IL-6, TNF-α, and IL-10, with a further increase under MA exposure and water-restraint stress(Tomita et al., 2011). Both the Nfkbiz gene (a regulator of NF-κB) and the Nr4a1 gene (a transcription factor) are up-regulated by NF-κB signaling activation, which is associated with inflammatory response(Yamamoto et al., 2004, Shinone et al., 2010). Similarly, mRNA expression of Nfkbiz and Nr4a1 in the heart and TNF-α, IL-1β, and IL-6 levels in serum are significantly induced in mice after MA exposure (30 mg/kg), with further increases in TNF-α and IL-6 when the mice are restrained after MA administration(Shinone et al., 2010). These studies indicate that inflammation plays a key role in myocardial damage induced by MA, which can be aggravated by additional environmental stimuli. In the present study, IL-6 increased and IL-10 decreased after MA administration, but these changes were reversed by CBD (40 or 80 mg/kg), suggesting that CBD may have an anti-inflammatory protective effect on myocardial damage induced by MA (Fig. 4A and 4B).

Previous research has indicated that CBD treatment (2 μg/μl) can inhibit the increase in IL-1β mRNA expression in the prefrontal cortex of rats following MA exposure(Karimi-Haghighi et al., 2020). Furthermore, CBD treatment (1.5 mg/kg, IP, 10 weeks) can significantly decrease pro-inflammatory cytokine IL-23, its receptor, CXCL-9, and CXCL-11 in mice with spinal cord injury, but not IL-6 or INF-γ(Li et al., 2018). In rats with myocardial ischemic reperfusion injury, CBD (5 mg/kg, IP, 7 days) can reduce infract size, myocardial inflammation, and serum IL-6(Durst et al., 2007). Myocarditis, focal and diffuse myocardial fibrosis, and myocardial dysfunction are reported in patients with pheochromocytoma, indicating that catecholamine toxicity may lead to myocarditis and myocardial fibrosis(Ferreira et al., 2016). This is supported by our study, whereby MA induced a cardiac inflammatory response and myocardial fibrosis, but these effects were attenuated by CBD in a dose-dependent manner (Fig. 3 and Fig. 4). Thus, CBD exhibited considerable preventive and therapeutic effects against cardiac damage induced by MA exposure, which may be mediated by a reduced inflammatory response.

To evaluate the extent of myocardial necrosis caused by MA, we detected cTnI levels in the left ventricle using western blot analysis, as shown in Fig. 4C. Creatine kinase myocardial band (CK-MB) is a key biomarker of myocardial infarction. Autopsy studies have shown high cTnI expression in fatal MA abusers, with CK-MB levels also increased in cardiac and peripheral blood(Zhu et al., 2007) and pericardial and cerebrospinal fluids of MA abusers(Wang et al., 2011). Elevated levels of cTnI and CK-MB are indicative of increased myocardial necrosis, as found in our study following MA administration. However, we also found that CBD (80 mg/kg) pretreatment decreased cTnI levels compared to the MA group, indicating that high-dose CBD may have a protective effect on cardiac damage. Similar findings have been reported in rabbits with acute myocardial infraction, with CBD administration (100 μg/kg) significantly decreasing plasma levels of cTnI and reducing ischemic injury in the myocardium(Feng et al., 2015).

The distribution of MA in the major organs of MA-sensitized rats is reported to be higher in the brain and heart than in the kidney, blood, and abdominal muscle, and delayed efflux of MA in the heart may be associated with cardiac toxicity(Nakagawa et al., 2003). The brain corticotrophin releasing factor system, which is associated with cardiac sympathetic control, is activated by chronic MA administration and withdrawal in mice, which further activates the sympathetic pathways in the heart with increased levels of phospho-tyrosine hydroxylase (p-TH) and p-heat shock protein 27 (p-HSP 27), which may be the mechanism of cardiovascular risk related to MA abuse(Garcia-Carmona et al., 2018). Although various pathological mechanisms have been investigated, the mechanism underlying myocardial injury caused by MA remains unclear. Our data showed that the PKA/CREB signaling pathway was activated and p-PKA and p-CREB increased in rats under chronic MA administration. These findings suggest that the PKA/CREB pathway participated in MA-induced myocardial inflammation and myocardial pathology. Increased cellular cAMP promotes the dissociation of PKA, the catalytic subunit of which migrates to the nucleus and phosphorylates CREB at a single phospho-acceptor site (ser 133), with p-CREB then promoting further transcription(Mayr and Montminy, 2001). PKA is the key kinase for CREB phosphorylation(Meyer et al., 2000), and CREB plays an important role in drug addiction(Zhou and Zhu, 2006). PKA, p-PKA, CREB, and p-CREB are highly expressed in different brain regions of MA-induced

conditioned place preference (CPP) rats and in SH-SY5Y cells, but can be inhibited by gastrodin(Yang et al., 2020b). The cAMP/PKA/CREB pathway is also involved in the apoptosis of cortical neurons induced by MA, but can be regulated by the neuroprotective effects of gastrodin(Ma et al., 2020). In this study, the expression levels of PKA, p-PKA, CREB, and p-CREB decreased following CBD pretreatment, indicating that CBD may attenuate myocardial inflammation and cardiac pathology by mediating the PKA/CREB signaling pathway. Similarly, CBD has shown potential therapeutic effects on MA-induced CPP in rats via the PI3K/AKT/GSK-3β/CREB signaling pathway(Yang et al., 2020a).

To the best of our knowledge, this study is the first to report on the protective effects of CBD on cardiac pathology elicited by chronic MA exposure in rats, with inhibition of cardiomegaly and reversal of histopathology, inflammatory response, and necrosis. Results showed that CBD at 40 and 80 mg/kg had a protective effect on MA-induced cardiac damage, although the effect was stronger at the higher concentration (80 mg/kg). These findings are similar with previous study showing that CBD at 80 mg/kg, but not 40 mg/kg, can reduce motivation of self-administered MA and drug-seeking behavior after extinction(Hay et al., 2018). CBD may exhibit cardioprotective effects by modulating the expression of crucial components of the cAMP/PKA/CREB signaling pathway. Our study highlights the potential clinical application of CBD in MA-induced cardiac pathology.

Our study demonstrated that chronic MA administration induced cardiomegaly and cardiac pathology in rats, with a notable increase in inflammatory response and myocardial necrosis. Interestingly, CBD pretreatment significantly and dose-dependently reduced the inflammatory response and myocardial necrosis via regulation of the PKA/CREB signaling pathway. These results indicate that CBD may have potential clinical application for the treatment of MA-induced cardiotoxicity. However, the specific molecular mechanism of MA-induced cardiotoxicity and the protective effects of CBD need to be further investigated.

Declarations

Author Contributions

The authors declare that all data were generated in-house and that no paper mill was used. S.H. and L.L. were responsible for overall direction of the project. Q.N. performed experiments and drafted the manuscript. W.D. and B.S. performed daily injections of methamphetamine and cannabidiol. G.Y. and H.Y. performed experiments. R.Z. and Y.P. collated data. Y.Y. performed data analysis. All authors read and approved the final manuscript.

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Availability of data and materials

Data and figures are available upon request.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

All animal studies were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of KMU, and all experimental procedures were approved by the Ethics Committee on Animal Care and Use from KMU (Approval code: kmmu2020403).

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

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Figures

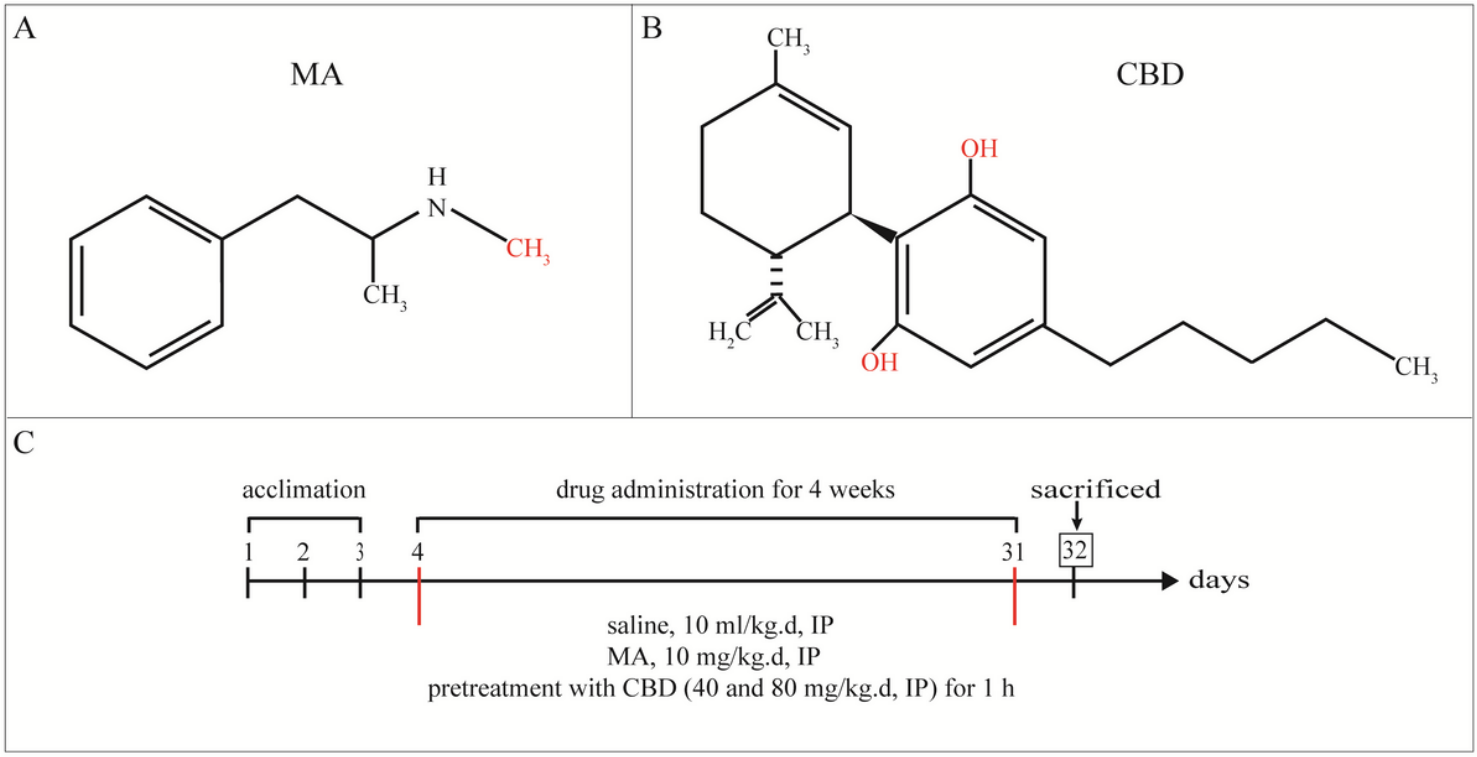

Figure 1

Experimental design for CBD and MA treatments in rats. (A) Chemical structure of MA. (B) Chemical structure of CBD. (C) Acclimation phase (days 1–3). Drug administration was once a day over 4 weeks (days 4–31). Rats in MA group received MA (10 mg/kg) by intraperitoneal injection (IP). CBD_{40} + MA group and CBD_{80} + MA group rats received MA (10 mg/kg) following 1-h CBD treatment (40 or 80 mg/kg, IP). Control group rats received saline solution (containing 5% DMSO + 5% Tween-80, 10 ml/kg, IP). Rats in CBD_{80} group received CBD only (80 mg/kg, IP). On day 32, all rats were sacrificed for collection of hearts and cardiac blood for further analysis.

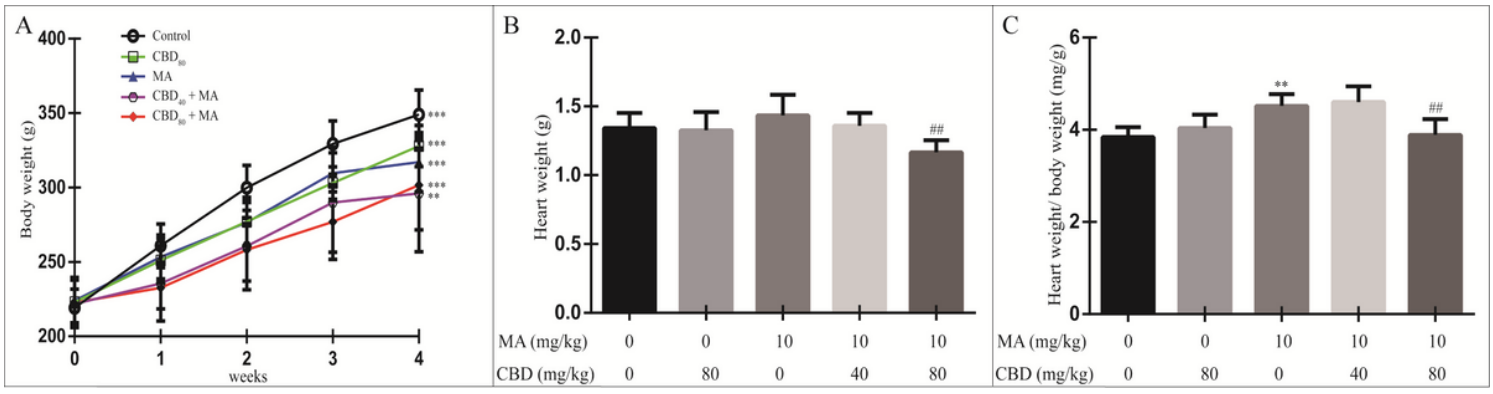

Figure 2

Effects of CBD on MA-induced body and heart weight changes in rats. Rats were pretreated with CBD (40 or 80 mg/kg, IP) for 1 h before administration of MA (10 mg/kg, IP), once a day for 4 weeks. (A) Effects

of CBD on MA-induced body weight changes. Data are mean ± SD, and paired *t*-tests were applied to evaluate body weight, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. before drug administration (0 week). (B) Effects of CBD on MA-induced heart weight gain. Data are mean ± SD, $^{\#\#}P < 0.01$ vs. MA group. (C) Effects of CBD on MA-induced heart-to-body weight ratio increase. Data are mean ± SD, $^{**}P < 0.01$ vs. control group; $^{\#\#}P < 0.01$ vs. MA group.

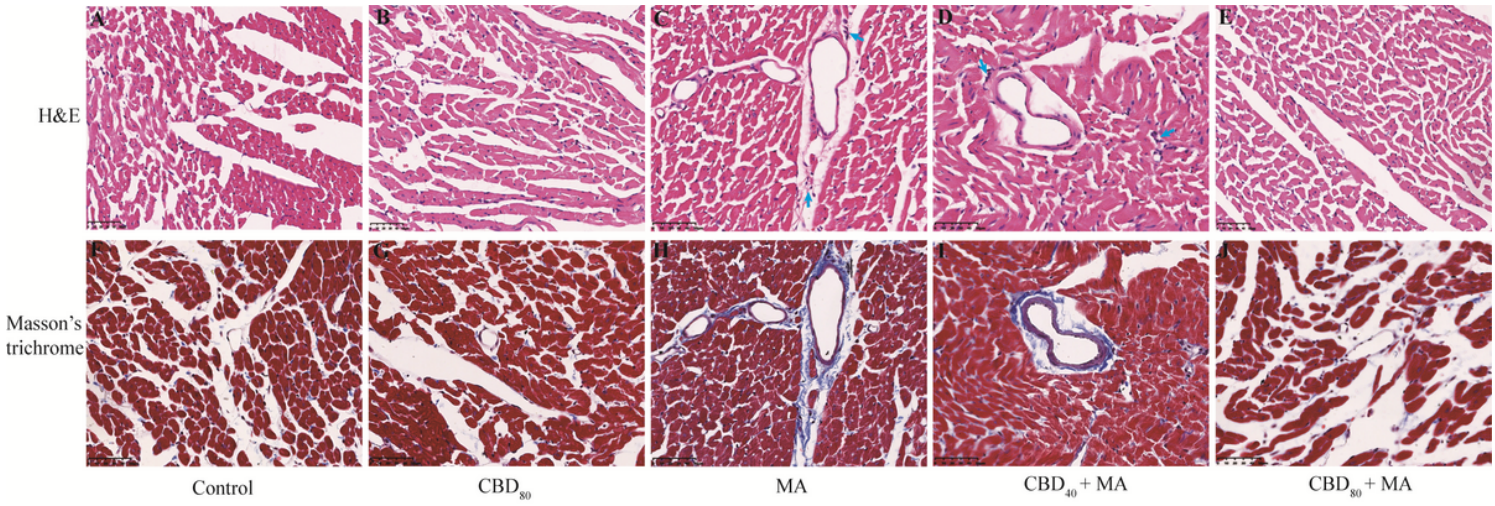

Figure 3

Effects of CBD on MA-induced cardiac histopathological changes. Rats were pretreated with CBD (40 or 80 mg/kg, IP) for 1 h before administration of MA (10 mg/kg, IP), once a day for 4 weeks. Images represent hematoxylin & eosin (H&E) and Masson's trichrome staining, respectively. Scale bar, 50 μm. (A) Control and (B) CBD_{80} groups showed normal myocardial tissue structure. (C) MA group showed myocardial fiber disorder, myocyte vacuolization, and mononuclear inflammatory infiltration with myocardial fibrosis or necrosis (blue arrow). (D) CBD_{40} + MA group showed less myocardial injury than MA group, but still exhibited mononuclear inflammatory infiltration with fibrosis or necrosis in interstitial spaces or around blood vessels (blue arrow). (E) CBD_{80} + MA group showed cardiac myocyte degeneration. Compared with (F) control group and (G) CBD_{80} group, (H) MA group showed blue-stained collagen fibrils in perivascular and interstitial spaces. (I) CBD_{40} + MA group showed less perivascular and interstitial fibrosis compared with MA group. (J) CBD_{80} + MA group showed almost no collagen fibrils in perivascular and interstitial spaces.

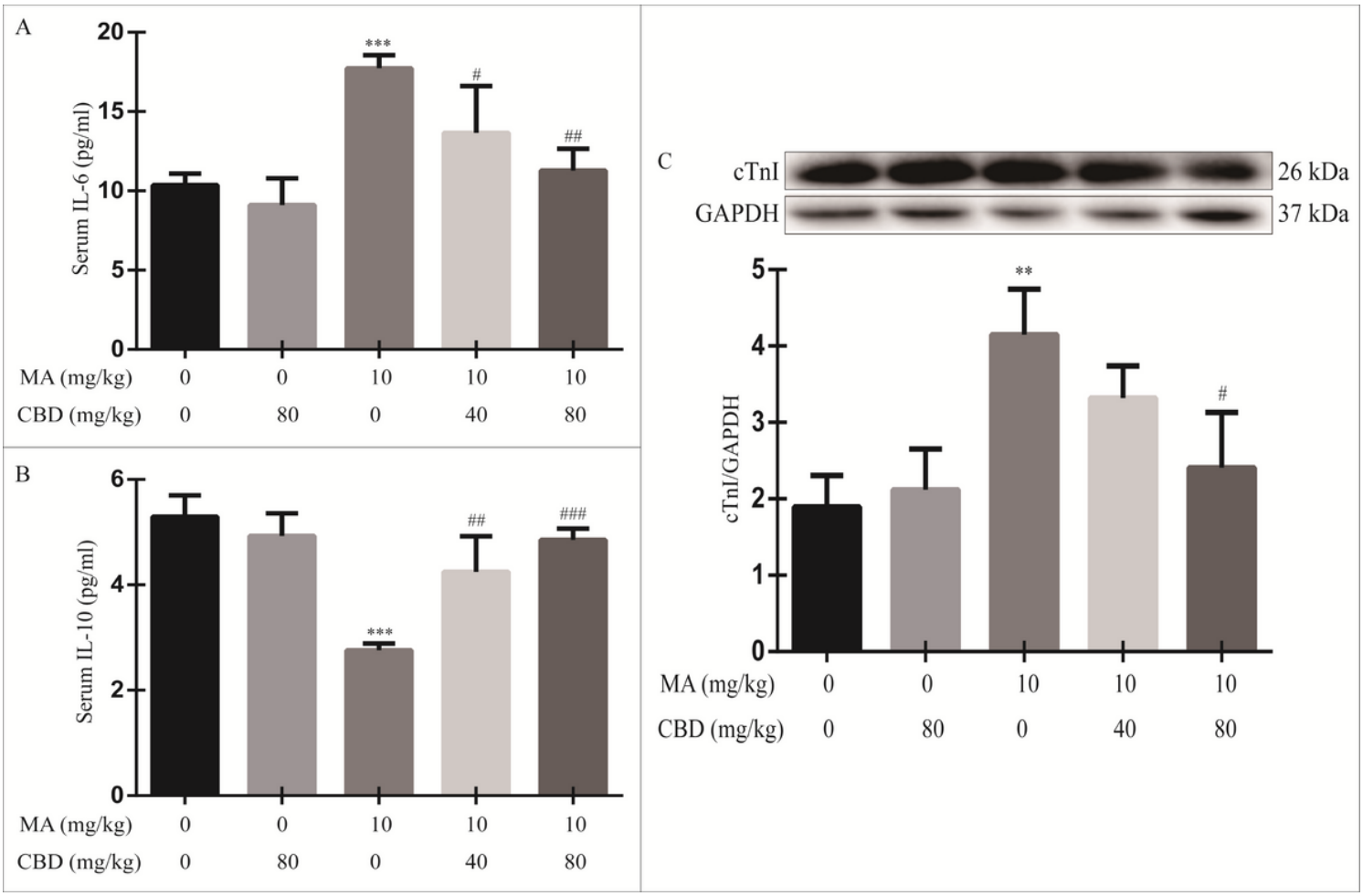

Figure 4

Effects of CBD on MA-induced cytokine and cTnI expression. Rats were pretreated with CBD (40 or 80 mg/kg, IP) for 1 h before administration of MA (10 mg/kg, IP), once a day for 4 weeks. Serum levels of IL-6 (A) and IL-10 (B) were detected by ELISA. (C) cTnI expression in left ventricle was measured by western blot analysis. Data are mean ± SD, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group; $^{\#}P < 0.005$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. MA group.

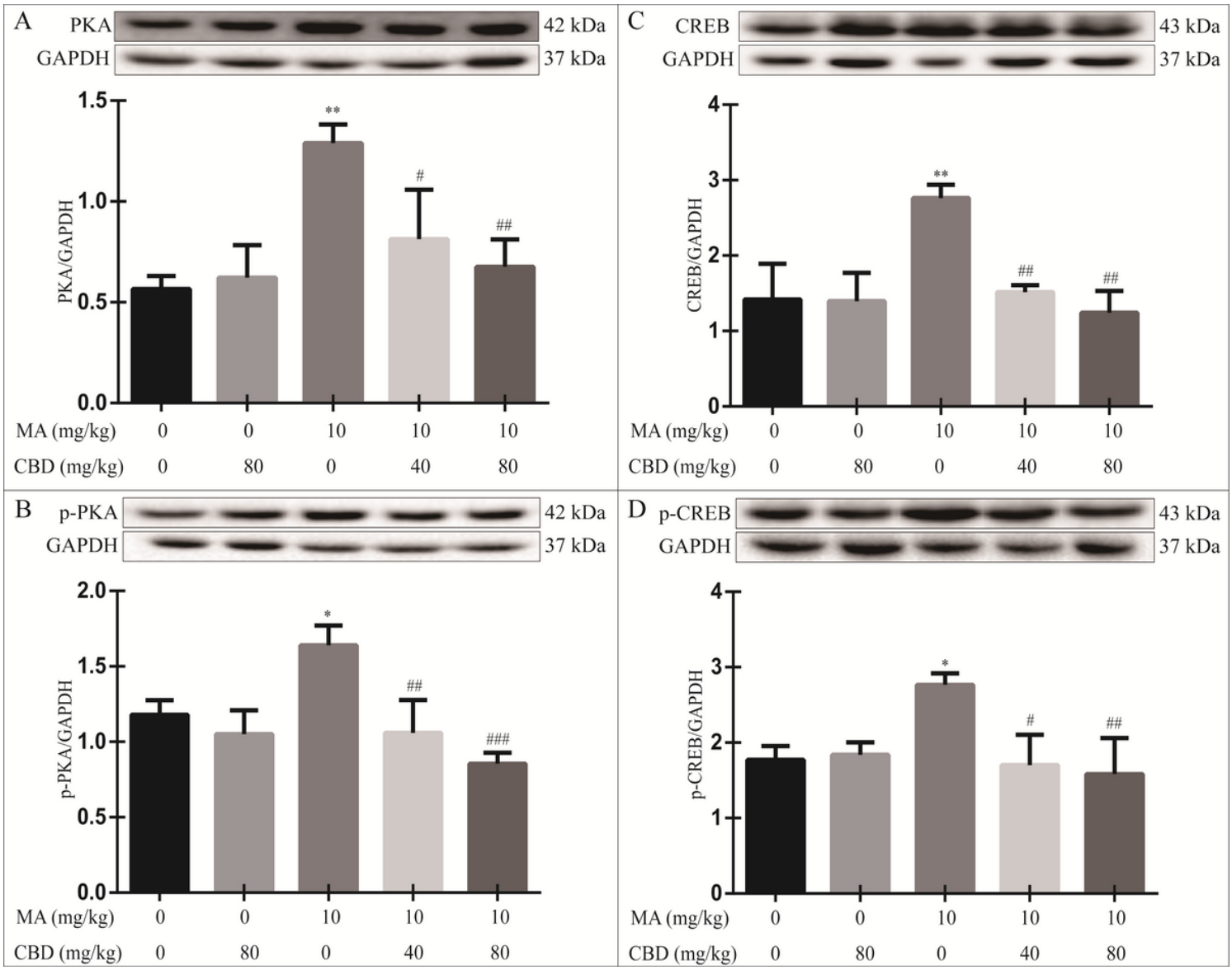

Figure 5

Effects of CBD on PKA, p-PKA, CREB, and p-CREB in left ventricle of MA-induced rats. Rats were pretreated with CBD (40 or 80 mg/kg, IP) for 1 h before administration of MA (10 mg/kg, IP), once a day for 4 weeks. Expression levels of PKA (A), p-PKA (B), CREB (C), and p-CREB (D) were measured by western blot analysis. Data are mean ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group; $^{\#}P < 0.005$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. MA group.

Supplementary Files

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