

The PKM2 activator TEPP-46 attenuates MCD feeding-induced nonalcoholic steatohepatitis by inhibiting the activation of Kupffer cells

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Abstract. – OBJECTIVE: The present study aimed to investigate the effect and molecular mechanism of the PKM2 small molecule agonist TEPP-46 on the development of methionine choline-deficient (MCD) diet-induced nonalcoholic steatohepatitis (NASH) in mice.

MATERIALS AND METHODS: In this study, C57BL/6 mice were fed an MCD diet for 15 days to establish a NASH model. The protein expression levels of pyruvate kinase M2 (PKM2), PKM1, hypoxia-inducible factor-1 α (HIF-1 α) and NLRP3 in liver Kupffer cells (KCs) were measured by Western blotting. Immunofluorescence analysis was used to analyze the nuclear translocation of PKM2 in KCs, and the levels of IL-1 β and TNF- α in mouse serum and the cell polarization indexes were determined. The MCD diet-fed mice were injected with 30 mg/kg of TEPP-46 intraperitoneally every 5 days. After 15 days, the liver tissue and peripheral blood were collected for analysis.

RESULTS: We found the NASH model was successfully established after the mice were fed an MCD diet for 15 days. MCD feeding promoted the expression of the PKM2 monomer/dimer and inhibited the expression of the PKM2 tetramer in KCs. Immunofluorescence analysis further confirmed that MCD feeding inhibited the nuclear translocation of PKM2. Besides, MCD feeding promoted the expression of HIF-1 α and NLRP3 in KCs, promoted M1 KCs polarization and inhibited M2 KCs polarization. Intraperitoneal injection 30 mg/kg of TEPP-46 significantly inhibited the development of MCD diet-induced NASH, alleviated the pathological changes in the liver, improved liver function, promoted the expression of the PKM2 tetramer in KCs, and inhibited the expression of HIF-1 α and NLRP3.

CONCLUSIONS: This study demonstrated that TEPP-46, a small molecule agonist of PKM2, may inhibit the nuclear translocation of PKM2 and the activation of KCs by promoting the expression of PKM2 tetramers in KCs, thus inhibiting the development of MCD diet-induced NASH in mice.

Key Words:

Nonalcoholic steatohepatitis, Pyruvate kinase M2, TEPP-46, Kupffer cells.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is defined as the presence of a significant amount of lipid accumulation in the liver without significant alcohol consumption¹. NAFLD represents two distinct conditions with two different prognoses, simple fat accumulation in the liver, and nonalcoholic steatohepatitis (NASH), which involves necrotic inflammation and may lead to fibrosis, cirrhosis and hepatocellular carcinoma (HCC)^{1,2}. Approximately 10%-20% of NAFLD cases can gradually develop into NASH³. NASH causes more serious liver steatosis and liver cell damage than NAFLD, which are closely related to the occurrence of liver cirrhosis and liver cancer^{2,4}. The pathophysiology of NASH is multifactorial and not yet completely understood; however, innate immunity is a major contributing factor, and liver-resident macrophages (Kupffer cells, KCs) and recruited macrophages play central roles in disease progression⁵. Under pathological conditions, KCs are activated and can promote the release of inflammatory mediators such as interferon (IFN), IL6, reactive oxygen species (ROS) and nitric oxide (NO) by activating the NLRP3 inflammasome, thus promoting the occurrence and development of NASH⁶. MCC950 is a selective NLRP3 inhibitor, improved NASH pathology and fibrosis in obese diabetic mice⁷. Macrophage-specific hypoxia-inducible factor 1 α (HIF-1 α) contributes

to impaired autophagic flux in NASH⁸. These studies show that the macrophage-mediated inflammatory response is an important link in the progression of NASH.

Pyruvate kinase M2 (PKM2) is a subtype of pyruvate kinase (PK), which includes PKM1, PKL and PKR, in addition to PKM2^{9,10}. PKM1 is mainly expressed in myocardial tissue, muscle and the brain¹¹. PKL and PKR are expressed in hepatocytes and erythrocytes, respectively^{10,12}. Although PKM2 and PKM1 differ by only 23 amino acid sequences, their functions are markedly different due to their structural differences¹³. PKM2 has three configurations in cells: monomeric, dimeric and tetrameric¹⁴. Monomers and dimers are mainly located in the nucleus and play important roles in regulating gene transcription, while tetramers play an important role in maintaining the normal energy metabolism of cells¹⁴. In normal cells, PKM2 mainly exists as tetramers, and in most tumor cells, PKM2 exists as mono/dimers, which can promote the development of tumors by directly binding with HIF-1 α , inducing the cells to produce energy by glycolysis¹⁵. Macrophages also contain PKM2 mono/dimers in the inflammatory state, that is, the transition from tetramers to mono/dimers (nuclear translocation) occurs¹⁶. The secretion of inflammatory factors by activated macrophages and M1 macrophage polarization are closely related to PKM2 nuclear translocation¹⁷. Inhibition of PKM2 nuclear translocation by using the small molecule agonist DASA-58 or TEPP-46 and the promotion of PKM2 tetramer expression can significantly inhibit the macrophage-mediated inflammatory response^{16,18}.

NASH is also an inflammatory process of the liver that is closely related to the activation of KCs⁵. At present, it is unclear how PKM2 is expressed in KCs and how PKM2 affects NASH development. Therefore, in this study, the role and molecular mechanism of PKM2 in NASH development were investigated. In this study, we used methionine choline-deficient (MCD) diet-fed C57BL/6 mice to establish a NASH model and treated the mice with TEPP-46 by intraperitoneal injection to explore the changes in PKM2 expression in mouse hepatocytes and KCs during MCD feeding and the influence of TEPP-46 on the development of MCD diet-induced NASH and its molecular mechanism, thus confirming the role and molecular mechanism of PKM2 in KCs in the development of NASH.

Materials and Methods

Mice and NASH Model

Male C57BL/6 mice (8-week-old, 22-25 g) were obtained from the Animal Experimental Center of Chongqing Medical University. All mice were fed sterile water ad libitum and housed at 22°C with 50% humidity and 12 h light/dark cycles. All the mice were divided into three groups and 10 mice in each group (control group, MCD group and TEPP-46+MCD (TEPP-46) group). The control group was fed general food, the MCD group was fed MCD (Dyets Inc., Bethlehem, PA, USA) feed for 15 days, and the TEPP-46 group was fed MCD feed and injected with 30 mg/kg of TEPP-46 (HY-18657, MedChemExpress, NJ, USA) by intraperitoneal injection every 5 days. After 15 days of feeding, mice were deeply anesthetized by inhaling isoflurane. Then, the left eyeball of the mouse was removed, and the peripheral blood of the mouse was collected from posterior frame venous plexus (about 1 ml blood of each mice). After that, liver tissues were collected from the mice for follow-up analysis. This study was approved by the Ethics Committee of Tongnan District People's Hospital (No. 2017-36).

KCs Isolation, Purification and Culture

The isolation, purification and culture of KCs were performed as previously described¹⁹. In short, anesthesia, fixation, laparotomy, perfusion, liver harvesting, liver homogenization, digestion, gradient centrifugation, and culture were performed. The mice were anesthetized, fixed on an animal operating table, and sterilized, and the abdominal cavity was opened layer by layer. Expose the portal vein of the liver. 10 ml of type IV collagenase (0.5%) was slowly perfused into the liver through the portal vein. The liver tissue was removed and quickly homogenized in culture medium containing collagenase until the liver tissue was fine and granular. Then, the liver tissue suspension was placed into a 15 ml sterile centrifuge tube and incubated at 37°C for 30 min to fully digest the liver tissue. Then, KCs with a high purity were separated by gradient centrifugation. The initially obtained KCs contained many red blood cells, hepatocyte, neutrophils, etc. Because red blood cells, hepatocytes and neutrophils were nonadherent and KCs were adherent, the cells were cultured for 2 h, and then, the solution was changed to remove the non-adherent cells. After the KCs were cultured for 24 h, an appropriate amount of F4/80 antibody (F4/80 is a macrophage marker) was added, and

then an appropriate amount of secondary antibody was added. The purity of KCs was detected by flow cytometry. The positive rate of F4/80 cells is about 70%, which indicates that the purified KCs have high purity.

Western Blotting

Total proteins were extracted from KCs by using a total protein extraction kit (KGP250, KeyGEN, Nanjing, China). The concentration of protein was determined by BCA protein assay kit (P0012s, Beyotime, Shanghai, China). The protein samples were mixed with 5× loading buffer (the protein samples used to detect PKM2 were mixed with nondenaturing loading buffer without SDS and were not denatured by boiling) at a volume ratio of 4:1 and boiled in water for 10 min. Then, the proteins were resolved by SDS-polyacrylamide gel electrophoresis (SDS-free gels for PKM2 analysis) and transferred to polyvinylidene difluoride (PVDF) membranes, which were blocked with 5% bovine serum albumin (BSA) for 1 h at room temperature. The membranes were then incubated overnight at 4°C with primary antibodies against PKM2 (1:1,000; #4053; Cell Signaling Technology, Inc.), PKM1 (1:1,000; #7067; Cell Signaling Technology, Inc. Danvers, MA, USA), HIF-1 α (1:1,000; ab86299; Abcam, Cambridge, MA, USA), NLRP3 (1:1,000; ab179483; Abcam) and β -actin (1:1,000; ab179467; Abcam). The membranes were further incubated with the corresponding secondary antibody (1:10,000; #7074/#7076; Cell Signaling Technology, Inc.) at 37°C for 1 h. After being incubated with peroxidase-conjugated secondary antibodies, the PVDF membranes were washed with TBST 3 times, incubated with enhanced chemiluminescence reagent (#32106, Pierce, Rockford, IL, USA) for 10 sec, and then analyzed and scanned using Quantity One software (Bio-Rad).

RT-qPCR Analysis of Inflammatory Factors and Polarization Indicators

Total RNA was extracted from KCs using TRI reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. Then, the total RNA was reverse transcribed to cDNA with the PrimeScript™ RT reagent kit (Takara Bio, Tokyo, Japan). Finally, RT-qPCR was performed. The gene expression levels were normalized to that of GAPDH. The primer sequences were as follows: cIAP1, forward: 5'-CACTAC-CCCACCCCACTC-3', reverse: 5'-AACGGAG-CAAGACCCTGT-3'; CD11c, forward: 5'-CT-

GTTGCTGCTGGCACTC-3', reverse: 5'-GG-GGTCTGTGTCCCGTT-3'; iNOS, forward: 5'-CACCTGGGGTTCTTGGG -3', reverse: 5'-CTGGGAGTTGGGGTGATG-3'; TNF- α , forward: 5'-CCCTCCTTCAGACACCCT-3', reverse: 5'-GGTTGCCAGCACTTCACT -3'; IL-1 β , forward: 5'-TTGAGTCTGCCCAGTTCC-3', reverse: 5'-TTTCTGCTTGAGAGGTGCT-3'; and GAPDH, forward: 5'-CCTTCCGTGTCCCCACT-3' and reverse: 5'-GCCTGCTTCACCACCTTC-3'.

Analysis of Serum Cytokines and Liver Function

The serum inflammatory factors TNF- α and IL-1 β and liver function indexes ALT, AST and TBIL were measured by the Animal Experimental Center of Chongqing Medical University.

Immunofluorescence Analysis of PKM2 in KCs

KCs were inoculated into 24-well plates containing climbing plates and 1 ml of culture medium in each well. After 4 h, the cells were washed with precooled PBS 3 times. Then, the cells were fixed with 4% paraformaldehyde at room temperature for 15 min, and the cell membrane was permeabilized with 0.5% Triton for 10 min. Then, the cells were blocked with goat serum for 1 h. The PKM2 antibody (1:1,000; #4053; Cell Signaling Technology, Inc. Danvers, MA, USA) was added and incubated overnight at 4°C. The next day, the climbing tablets were washed with PBS 3 times and then incubated with a fluorescent secondary antibody (ZSGB Bio, Beijing, China) for 1 h. After washing the climbing tablets, an appropriate amount of DAPI was added to each climbing plate for 3 min, and the plates were washed again. The tablets were sealed with an anti-fluorescence quencher. Then, the protein expression of PKM2 was observed under an inverted microscope.

Immunohistochemical Analysis of PKM2 in Liver Tissues

Immunohistochemical staining was performed as previously described²⁰. In short, the liver tissues were fixed, embedded, sliced, dewaxed, rehydrated and subjected to antigen retrieval. Then, the slides were blocked with 5% goat serum (Beyotime) at room temperature for 1 h. Next, the slides were incubated with the PKM2 antibody (1:1,00; #4053; Cell Signaling Technology, Inc. Danvers, MA, USA) at 4°C overnight. The next day, the slides were incubated with a secondary

antibody for 1 h. Then, after being stained with DAB solution (Boster) and hematoxylin, the sections were dehydrated and sealed with neutral gum. Finally, the expression of PKM2 was observed using an upright light microscope.

Hematoxylin-eosin (HE) Staining

HE staining of mouse liver tissue was completed by the Pathology Department of Chongqing Medical University.

Statistical Analysis

Statistical analyses were performed using SPSS 17.0 software (SPSS, Inc.). Student's *t*-test was used for the comparison of parameters between two groups. One-way ANOVA and Tukey's test were used to compare the parameters among >2 groups. A value of $p < 0.05$ was considered statistically significant.

Results

MCD Feeding Induces NASH Development in Mice

MCD feeding is the most common method used to establish a mouse/rat NASH model²¹. In our study, compared with normal feeding (control group), after 15 days of MCD feeding (MCD group) (Figure 1A), the weights of the mice and the liver volumes were significantly reduced (Figure 1B and C). HE staining showed that the liver tissue structure was disordered and exhibited vacuole-like changes, accompanied by a large amount of inflammatory cell infiltration in mice in the MCD group (Figure 1D). The levels of serum ALT, AST and TBIL and the inflammatory factors IL-1 β and TNF- α increased significantly in the MCD group compared with the control group (Figure 1E and F).

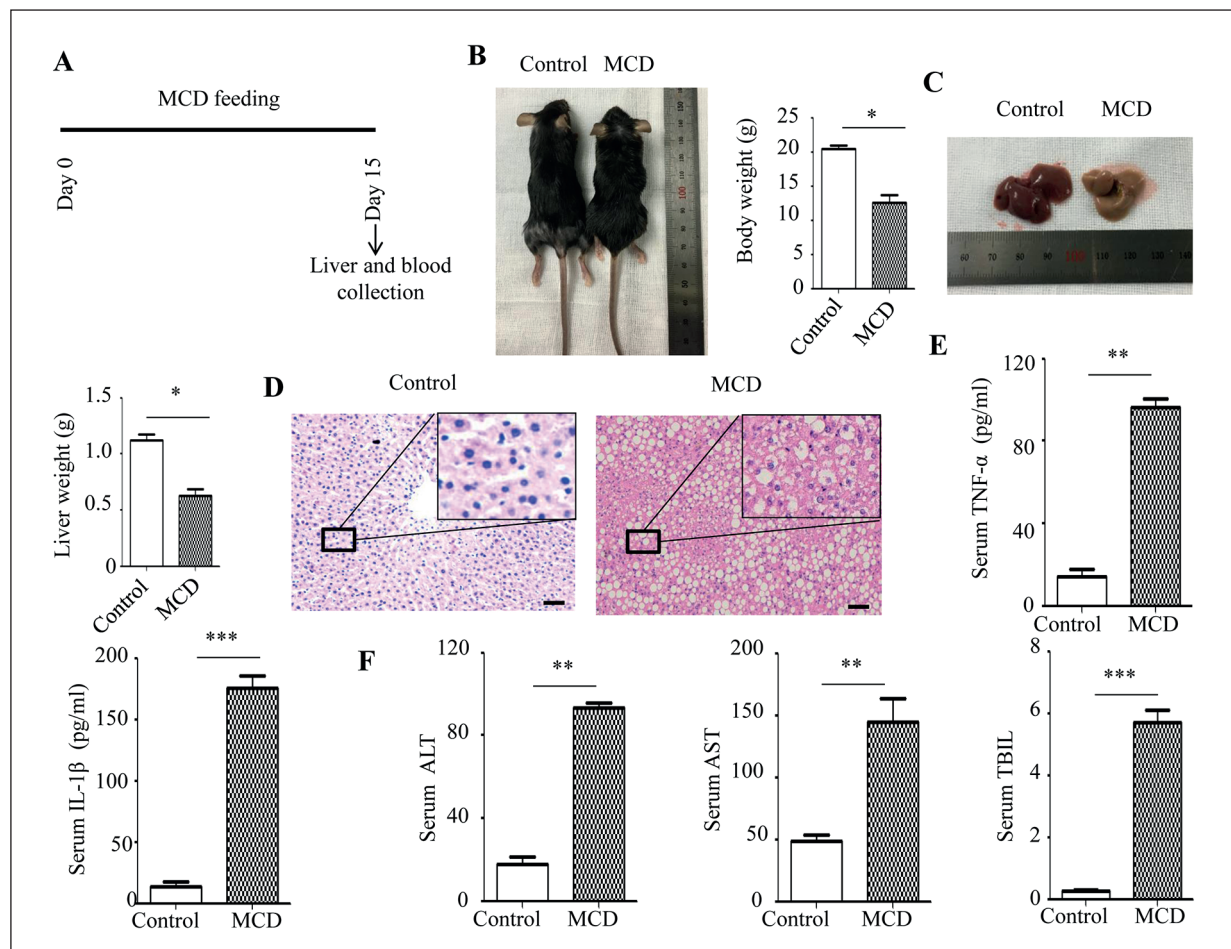


Figure 1. MCD diet induced NASH development. **A**, Schematic diagram of the model. **B**, Mouse morphology and weight. **C**, Liver morphology and weight. **D**, HE staining showing the pathological changes in the liver. (Scale bar: 1 mm) **E**, Serum TNF- α and IL-1 β levels. **F**, Serum ALT, AST and TBIL levels. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

These results suggested that the NASH model was successfully established after 15 days of MCD feeding.

MCD Feeding Promotes PKM2 Nuclear Translocation and NLRP3 Inflammasome Activation in KCs

KCs are innate immune cells in the liver that play an important role in regulating the liver immune response²². Research shows that the development of MCD diet-induced NASH in mice is related to the activation of KCs⁵. However, the specific molecular mechanism is still unclear. PKM2 shows different functions according to its different conformations. At present, it is unclear what changes occur in PKM2 in KCs and how PKM2 conformational changes affect MCD diet-induced NASH development in mice. There-

fore, this study further examined this problem. We extracted hepatocytes and KCs from mice in the control group and MCD group and measured the protein expression of PKM2 by Western blotting. MCD feeding had no significant effect on the expression of PKM2 in hepatocytes, while MCD feeding inhibited the expression of the PKM2 tetramer in KCs and promoted the expression of its monomer/dimer (nuclear translocation) (Figure 2A). Cellular immunofluorescence further confirmed that MCD feeding promoted the nuclear translocation of PKM2 (Figure 2B). Immunohistochemical analysis of PKM2 in liver tissue suggested that MCD feeding promoted PKM2 expression in KCs (Figure 2C). As the development of NASH is closely related to the activation of KCs, we further measured the expression of PKM1, HIF-1 α and NLRP3. We found

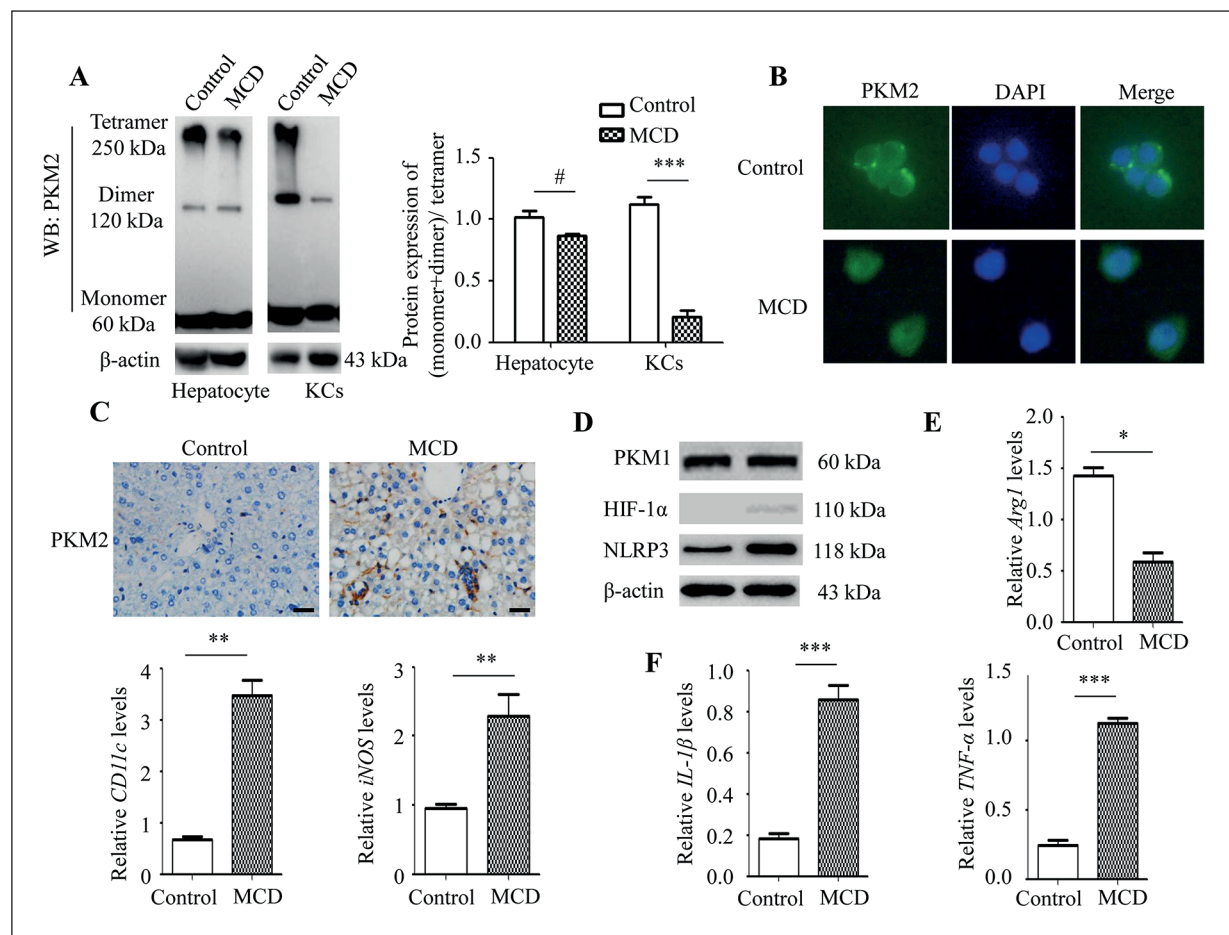


Figure 2. PKM2 expression in MCD diet-induced mice. **A**, Western blotting analysis of PKM2 protein expression levels in hepatocytes and KCs. **B**, Immunofluorescence analysis of PKM2 protein expression levels in KCs ($\times 200$). **C**, Immunohistochemical analysis of PKM2 protein expression in liver tissues (Scale bar: 1 mm). **D**, Western blotting analysis of the protein expression levels of PKM1, HIF-1 α and NLRP3 in KCs. **E-F**, qRT-PCR measurement of the mRNA levels of Arg1, CD11c, iNOS, TNF- α and IL-1 β in KCs. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

that MCD feeding did not affect PKM1 expression in KCs but promoted HIF-1 α expression and NLRP3 activation (Figure 2D). CD11c and iNOS are markers of M1 polarization, and Arg1 is a marker of M2 polarization of macrophages. MCD feeding promoted M1 and inhibited M2 polarization of KCs (Figure 2E). Besides, MCD feeding promoted the mRNA expression of TNF- α and IL-1 β (Figure 2F). These results suggest that MCD feeding may promote the activation of NLRP3 and the expression of inflammatory factors by promoting the nuclear translocation of PKM2 and induce NASH development.

The PKM2 Activator TEPP-46 Attenuates MCD Diet-Induced NASH

TEPP-46 is a specific small molecule agonist of PKM2 that can significantly inhibit the nuclear translocation of PKM2 and induce the formation of the PKM2 tetramer²³. To further explore the role of PKM2 in the development

of MCD diet-induced NASH, we established a mouse NASH model, as shown in Figure 3A. There were three groups: the control group, MCD group and TEPP-46+MCD (TEPP-46) group. The control group was fed general food, the MCD group was fed MCD feed for 15 days, and the TEPP-46 group was fed MCD and intraperitoneally injected with 30 mg/kg of TEPP-46 every 5 days. After feeding for 15 days, peripheral blood and liver tissues were collected from the mice for follow-up experiments. The masses and liver volumes of mice in the MCD group were significantly lower than those in the control group, while the masses and liver volumes of mice in the TEPP-46 group were significantly higher than those in the MCD group (Figure 3B and C). The weights and liver volumes of TEPP-46 mice were lower than those of control mice, but there was no significant difference between the two groups (Figure 3B and C). HE staining also showed that the degree of vacuole-like changes in the livers

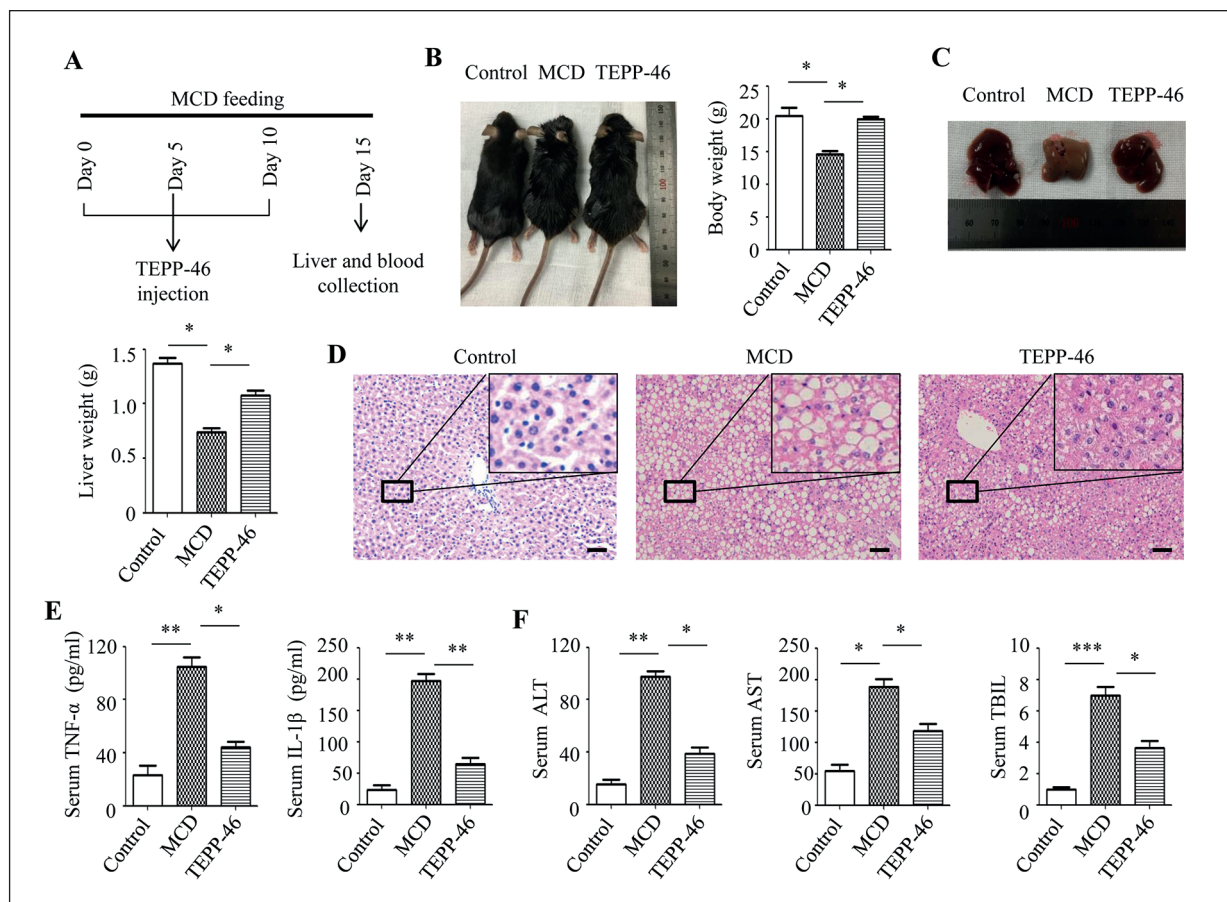


Figure 3. TEPP-46 attenuates MCD diet-induced NASH. **A**, Schematic diagram of the model. **B**, Mouse morphology and weight. **C**, Liver morphology and weight. **D**, HE staining showing the pathological changes in the liver. (Scale bar: 1 mm) **E**, Serum TNF- α and IL-1 β levels. **F**, Serum ALT, AST and TBIL levels. * p <0.05, ** p <0.01, *** p <0.001.

of mice in the TEPP-46 group was significantly lower than that in the MCD group, and there was no obvious inflammatory cell infiltration (Figure 3D). The levels of serum ALT, AST and TBIL and the inflammatory factors IL-1 β and TNF- α were significantly lower than those in the MCD group (Figure 3E and F). These results suggest that TEPP-46, a small molecule agonist of PKM2, can inhibit the development of MCD diet-induced NASH in mice.

TEPP-46 Inhibits the Activation of KCs by Promoting the Formation of the PKM2 Tetramer

To further explore the molecular mechanism by which TEPP-46 inhibits MCD diet-induced NASH development, we extracted KCs from mice in each group and measured the protein expression of PKM2 by Western blotting. MCD feeding promoted the formation of the PKM2 monomer (nuclear translocation), while TEPP-46 inhibited

the formation of the PKM2 monomer and promoted the formation of the PKM2 tetramer in KCs (Figure 4A). Immunohistochemical analysis of PKM2 in the liver tissue showed that TEPP-46 inhibited MCD diet-induced PKM2 expression (Figure 4B). In addition, TEPP-46 inhibited the MCD diet-induced expression of HIF-1 α and NLRP3 (Figure 4C). TEPP-46 also inhibited M1 polarization and promoted M2 polarization of KCs induced by MCD feeding (Figure 4D). TEPP-46 also inhibited the MCD diet-induced expression of IL-1 β and TNF- α in KCs (Figure 4E). These results indicate that the development of MCD diet-induced NASH may be closely related to the PKM2 nuclear translocation-mediated activation of KCs. Inhibition of PKM2 nuclear translocation by the PKM2 agonist TEPP-46 effectively inhibited the activation of KCs, inhibited the activation of the NLRP3-mediated inflammatory pathway, and further inhibited the development of NASH.

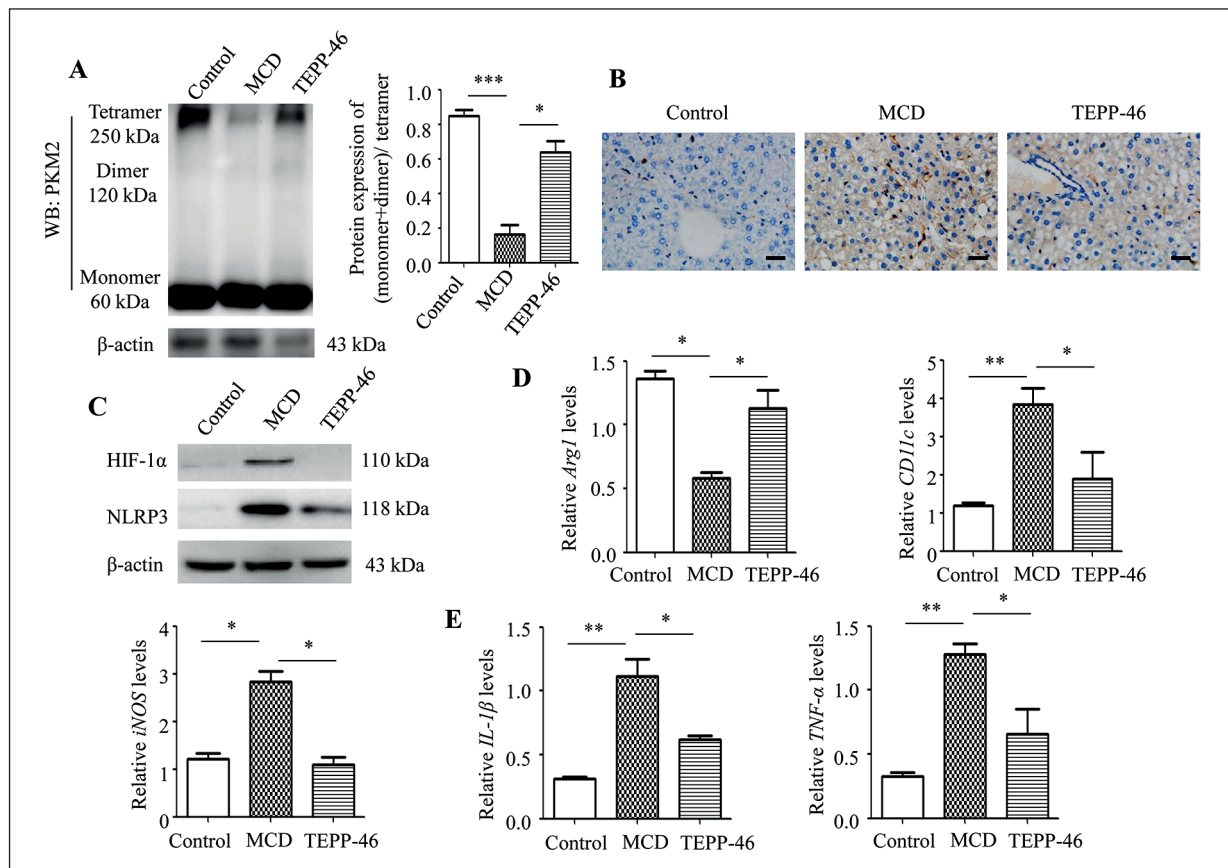


Figure 4. TEPP-46 inhibits the activation of KCs. **A**, Western blotting analysis of PKM2 protein expression levels in KCs. **B**, Immunohistochemical analysis of PKM2 protein expression in liver tissues. (Scale bar: 1 mm). **C**, Western blotting analysis of the protein expression levels of HIF-1 α and NLRP3 in KCs. **D-E**, qRT-PCR measurement of the mRNA levels of ARG1, CD11c, iNOS, TNF- α and IL-1 β in KCs. * p <0.05, ** p <0.01, *** p <0.001.

Discussion

The incidence of NAFLD is increasing each year and is closely related to the occurrence of hypertension, diabetes and various cardiovascular diseases^{1,2}. If NAFLD is not controlled, it may develop into NASH and then into liver fibrosis, cirrhosis and even hepatocellular carcinoma^{1,2}. At present, there have been many studies on NASH⁴. Although some achievements have been made, the mechanism of NASH occurrence and development is still unclear.

KCs are the innate immune cells in the liver and account for 80%-90% of all mononuclear cells/macrophages²². Studies have shown that the development of NASH is closely related to the overactivation of KCs in the liver⁵. Macrophages can be divided into the classically activated M1 type and selectively activated M2 type according to their phenotypes²⁴. Toxin or LPS can induce macrophages to M1-type polarization and promote the release of inflammatory factors in macrophages²⁴. Inducing M2 polarization of macrophages can inhibit LPS-mediated release of inflammatory factors^{14,24}. Macrophages with these two phenotypes differ greatly in function, which is closely related to the different energy metabolism modes of the cells¹⁴. PKM2 is the last rate-limiting enzyme in the glycolytic pathway, which catalyzes phosphoenolpyruvate and adenosine diphosphate (ADP) to produce pyruvate and adenosine Triphosphate (ATP)⁹. At present, PKM2 exists in three different conformations within cells: monomeric, dimeric and tetrameric¹⁴. To some extent, the function of PKM2 is determined by its conformational state⁹. Monomers and dimers are mainly located in the nucleus and play important roles in regulating gene transcription, while tetramers function as protein kinases, playing an important role in maintaining the normal energy metabolism of cells¹⁴. The function of PKM2 can be regulated by regulating the different conformations of PKM2, and cellular energy metabolism can then be regulated²⁵. Modulating the PKM2 conformation is an important link in the metabolic reprogramming of macrophages, and the transformation of PKM2 from the tetramer to the mono/dimeric form in macrophages activated by inflammation can promote the inflammatory reaction by inducing the transcription of HIF-1 α and high mobility group box 1 (HMGB1)²⁶. By inhibiting the translocation of PKM2 to the nucleus, the PKM2 agonist DASA-58 or TEPP-46

can promote the formation of the PKM2 tetramer and inhibit the inflammatory reaction²⁷. However, during the development of NASH, it is not clear how the expression of PKM2 changes, how the conformational change of PKM2 affects the development of NASH, and what the specific molecular mechanism is.

In this study, we successfully established a mouse NASH model by feeding mice with MCD diet for 15 days, which was mainly reflected in hepatic steatosis, inflammatory cell infiltration in liver lobules and balloon degeneration of hepatocytes. In fact, the duration of MCD feeding may be different in different studies. The short feeding time was only two weeks, and the long feeding time was 8 weeks or even 16 weeks^{28,29}. The longer the MCD feeding, the more severe the inflammation of the liver inflammation and the fibrosis^{28,29}. In this study, we found that MCD feeding promoted the expression of PKM2 in the mouse liver. By separating mouse hepatocytes and KCs, it was found that MCD feeding promoted the expression of PKM2 mono/dimers in KCs, inhibited the expression of PKM2 tetramers in KCs, and had no significant effect on the expression of PKM2 in hepatocytes. TEPP-46 is a small molecule inhibitor of PKM2 that can inhibit LPS-mediated macrophage activation and tumor cell growth by promoting the expression of the PKM2 tetramer^{15,16}. In this study, mice were fed an MCD diet and intraperitoneally injected with 30 mg/kg of TEPP-46 every 5 days. After 15 days, liver and peripheral blood were collected. TEPP-46 significantly inhibited the development of MCD diet-induced NASH, as indicated by the inhibition of vacuole-like changes in liver tissue and the inhibition of inflammatory cell infiltration. In addition, TEPP-46 also inhibited the serum levels of the proinflammatory factors IL-1 β and TNF- α and improved their liver function of mice. Many studies have shown that the development of MCD diet-induced NASH in mice is closely related to the overactivation of KCs in the liver³. We explored the molecular mechanism by which TEPP-46 inhibits the development of mouse NASH by extracting KCs from the mouse livers of each group. We found that TEPP-46 inhibited the MCD diet-induced activation of KCs, inhibited the expression of HIF-1 α and the NLRP3 inflammasome, inhibited the mRNA expression of IL-1 β and TNF- α in KCs, inhibited M1 KC polarization and promoted M2 KC polarization.

Conclusions

In summary, this study explored the effect of TEPP-46, a small molecule agonist of PKM2, on the development of MCD diet-induced NASH in mice and its molecular mechanism. However, there are some limitations in this study. For example, DASA-58 is also a small molecule agonist of PKM2. Whether DASA-58 has the same effect on the development of NASH in mice has not been studied. In addition, the research on the mechanism was not in-depth, and the results would be more convincing if we evaluated the effects at the gene intervention level, such as by establishing a targeted PKM2-knockout mouse.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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