

Discovery of CYP2E1 as a novel target in rheumatoid arthritis and validation by a new specific CYP2E1 inhibitor

Zixinying Han^a, Chenxu Liu^a, Mingrui Li^a, Mengyan Deng^a, Ying Ding^a, Yunchao Li^a, Meidan Huo^a, Haiwei Xu^b, Hailing Qiao^{a,*}, Na Gao^{a,*}

^a Institute of Clinical Pharmacology, School of Basic Medical Sciences, Zhengzhou University, Zhengzhou, Henan, China

^b School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou, Henan, China



ARTICLE INFO

Keywords:
CYP2E1
Rheumatoid arthritis
Specific inhibitor
ROS/Nrf2/HO-1

ABSTRACT

Considerable evidence indicates that CYP2E1 is associated with a variety of inflammatory diseases. Here we evaluated CYP2E1 as a potential therapeutic target for rheumatoid arthritis (RA) and established the protective effect of a new CYP2E1 inhibitor. Gene-expression datasets were used to analyze the change in expression of CYP2E1 in RA patients; CYP2E1 activity in collagen-induced arthritis (CIA) rats was determined by HPLC. We further evaluated the protective effects of *Cyp2e1* knockout and a CYP2E1-specific inhibitor, Q11, synthesized by our group, in CIA and adjuvant-induced arthritis (AIA) rats. The expression of CYP2E1 in synovial tissue was elevated in RA patients and in CIA rats and the activity of CYP2E1 in vivo and in vitro in CIA rats was greater than that of controls. *Cyp2e1* knockout significantly reduced the incidence of CIA and alleviated the severity of symptoms. Treatment with different doses of Q11 decreased paw thickness, volume and arthritis scores and reduced the serum levels of IL-6, TNF- α , IL-1 β and MDA, and increased the level of GSH in CIA rats. A similar inhibitory effect was exhibited for Q11 in the AIA rats. Moreover, Q11 significantly impeded proliferation, migration, and invasion of human rheumatoid arthritis synovial fibroblasts cells. Q11 decreased the release of ROS and enhanced Nrf2 nuclear translocation and HO-1 expression in the cell nucleus. Overall, our results indicated that CYP2E1 may be a new target for RA and Q11 has potential protective effects against RA by reducing oxidative stress and opposing the inflammatory response via the ROS/Nrf2/HO-1 signaling pathway.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, progressive inflammatory disorder, which in severe cases may result in permanent joint damage and disability [1,2]. RA is associated with increased mortality and occurs 2 to 3 times more frequently in women than in men [1,3]. A common first-line therapy is a weekly dose of methotrexate, which belongs to disease-modifying antirheumatic drugs (DMARDs). However, about 50 % patients with RA do not respond well to treatment with methotrexate [3]. For these patients, immunosuppressive medications should be used, such as tumor necrosis factor- α (TNF- α) inhibitors and Janus kinase inhibitors. Nonsteroidal anti-inflammatory drugs (NSAIDs) are

also used to treat RA but they do not prevent joint destruction. Moreover, adverse reactions to NSAIDs are common, including cardiovascular risks [4]. Therefore, it is necessary to look for more effective targets and more effective drugs for RA.

The pathogenesis of RA remains elusive. It is currently believed that inflammation and oxidative stress play essential pathological roles in the occurrence and development of RA [5–7]. Normally, reactive oxygen species (ROS) are produced in a controlled manner and physiological levels of ROS play an important role in maintaining normal cellular functions and physiological defense, while excess ROS can trigger oxidative stress. Mitochondrial ROS production is significantly higher in whole blood and monocytes of RA patients than in healthy subjects [8].

Abbreviations: AIA, adjuvant-induced arthritis; ARE, antioxidant response element; CAT, catalase; CFA, complete Freund's adjuvant; CIA, collagen-induced arthritis; CYP, cytochrome P450; DMARDs, disease-modifying antirheumatic drugs; FLS, fibroblast-like synoviocytes; GEO, gene Expression Omnibus; GSH, glutathione-S-transferase; H&E, haematoxylin and eosin; MH7A, human rheumatoid arthritis synovial fibroblasts; Nrf2, nuclear factor erythroid 2-related factor 2; NSAIDs, Nonsteroidal anti-inflammatory drugs; RA, Rheumatoid arthritis; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF- α , tumor necrosis factor- α ; TR, turnover rate.

* Corresponding authors.

E-mail addresses: qiaohl@zzu.edu.cn (H. Qiao), gaonawei@zzu.edu.cn (N. Gao).

<https://doi.org/10.1016/j.bcp.2024.116501>

Received 31 January 2024; Received in revised form 11 August 2024; Accepted 20 August 2024

Available online 20 August 2024

0006-2952/© 2024 Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

ROS, including hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), hydroxyl radicals ($\cdot OH$), and singlet oxygen (1O_2), can degrade the joint cartilage and play a role as secondary messengers in RA inflammation [6]. It is well known that there is positive feedback between oxidative stress and inflammation. Antioxidant pathways consist of an enzymic antioxidant system and a nonenzymic antioxidant system [9], the former including catalase (CAT), glutathione-S-transferase (GSH), and superoxide dismutase (SOD). Notably, antioxidant enzymes such as GSH and SOD are significantly decreased in RA patients [10]. Therefore, suppression of oxidative stress and inflammation is an appropriate therapeutic strategy against RA [11].

The mitochondrial respiratory chain is the major source of ROS in the cell. In addition, cytochrome P450 (CYP) metabolism plays a significant role in ROS generation [12,13]. CYPs are divided into different families and subfamilies based on genetic similarity. ROS production may vary considerably depending on the CYP subtypes involved; compared with other CYP subtypes, CYP2E1 may generate more ROS [14]. The reason might be that the heme iron of CYP2E1 is constitutively in the high spin state, which is unique and facilitates electron transfer to dioxygen [15]. Recent studies have focused on the role of CYP2E1 in inflammatory disease. Several studies have indicated that CYP2E1 is a key target protein in the development of alcoholic and nonalcoholic fatty liver disease (FLD), the mechanism of which is related to a massive production of ROS [16]. The results of Diesinger et al showed that a specific CYP2E1 inhibitor reduced xenograft tumor growth in nude mice [17]. Cederbaum et al reported that CYP2E1 contributes to obesity-induced oxidant stress and liver injury [18]. Some studies have focused on CYP2E1 in extrahepatic tissues; Yu et al found CYP2E1 activity significantly increased following ischemia-reperfusion (I/R) in mice with increased ROS [19]. CYP2E1 has been associated with a variety of inflammatory diseases, but to our knowledge, no study thus far has addressed the role of CYP2E1 in the occurrence and development of RA.

Our group has been focused on the role of CYP2E1 in inflammatory disease, such as hepatocellular carcinoma and hepatic fibrosis [20–22]. In addition, our group developed a specific CYP2E1 inhibitor, 1-(4-methyl-5-thiazolyl) ethanone (Q11) with a K_i of 0.897 μM [23] and have demonstrated that it can be used to treat sepsis in mice by suppressing oxidative stress and NLRP3 activation [23]. In addition, our group also demonstrated that Q11 is a promising anti-inflammatory agent for glioblastoma treatment [24]. In this paper, gene-expression datasets were used to analyze the change in expression of CYP2E1 in RA patients; CYP2E1 activity in collagen-induced arthritis (CIA) rats was determined by HPLC. We further evaluated the protective effects of *Cyp2e1* knockout and the CYP2E1-specific inhibitor, Q11, in CIA and adjuvant-induced arthritis (AIA) rats. Human rheumatoid arthritis synovial-fibroblasts (MH7A) were selected to establish an inflammatory cell model to further explore the effects and mechanism of Q11 on RA.

2. Materials and methods

2.1. Chemicals and animals

Q11 (purity > 98 %), 1-(4-methyl-5-thiazolyl) ethanone, was synthesized by our laboratory. Freund's incomplete adjuvant and Type II collagen were supplied by Beijing Boleide Development of Science and Technology Co., LTD (China). Celecoxib was obtained from Shanghai Aladdin Biochemical Technology Co., Ltd (China). Chlorzoxazone (purity > 99.5 %) was supplied by the National Institute for Food and Drug Control (Beijing, China). 6-hydroxychlorzoxazone was purchased from Toronto Research Chemicals Inc (Canada). The MH7A cell lines were purchased from GuangZhou Jennio Biotech Co., Ltd (China).

Experiments were carried out on male Sprague Dawley (SD) rats (Experimental Animal Center of Zhengzhou University). *Cyp2e1* knockout rats were generated by a CRISPR/Cas9-based approach by Biocytogen Pharmaceuticals (Beijing) Co., Ltd. Briefly, two sgRNAs were designed by the CRISPR design tool (<https://cctop.cos.uni-heidelberg.de:8043>) to target a region upstream of exon 2 and downstream of exon 6. A T7 promoter sequence was added to the Cas9 or sgRNA template by PCR amplification in vitro. Cas9 mRNA and sgRNAs were co-injected into the cytoplasm of one-cell stage fertilized SD rat eggs. The injected zygotes were transferred into oviducts of pseudopregnant females to generate F0 rats. F0 rats with the expected genotype, confirmed by tail genomic DNA PCR and sequencing were mated with SD rats to establish a germline-transmitted F1 heterozygous rat. F1 heterozygous rats were genotyped by tail genomic PCR and DNA sequencing. Rats were housed under controlled environmental conditions with a 12 h light/dark cycle and fed standard laboratory chow and water. All animal experimental procedures in this study were approved by the Life Science Ethics Committee of Zhengzhou University.

2.2. The identification of gene expression profiles in rheumatoid arthritis patients

We retrieved gene-expression datasets related to rheumatoid arthritis, namely GSE206848 and GSE48780, from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>) for the purpose of conducting differential gene expression analysis. The downloaded platform and series of matrix files were converted using the R language software and annotation package. Probe IDs were mapped to international standard gene symbols and saved in a CSV file. Subsequent analysis of gene expression differentials was conducted utilizing the limma package within the Bioconductor framework (accessible online: <https://www.bioconductor.org/>). The related operating instruction codes were put into R, and the DEGs in synovial samples from rheumatoid arthritis patients and normal synovial samples of the two microarray datasets were analyzed by the limma software package.

2.3. Induction of Collagen-Induced arthritis (CIA) in rats

A CIA rat model was established according to the method described previously [25]. Briefly, an equal volume of type II collagen was emulsified in Freund's incomplete adjuvant by a high-speed homogenizer in an ice bath. CIA rats were immunized intradermally with 0.25 ml emulsion at the base of the tail. Seven days after the first immunization, equal amounts of emulsion were used as a booster. Typically, the first signs of arthritis appeared in this model at 3–7 days after the second immunization. The severity of arthritis of each foot was scored based on a 0–4 scale according to the criteria reported previously [26,27], where 0 = no evidence of erythema and swelling; 1 = erythema and mild swelling confined to the tarsals or ankle joint; 2 = erythema and mild swelling extending from the ankle to the tarsals; 3 = erythema and moderate swelling extending from the ankle; and 4 = erythema and severe swelling encompass the ankle, foot and digits, or ankylosis of the limb. If the score of four paws was greater than 4, the model was considered successful.

2.4. Changes in activity and content of CYP2E1 in CIA rats

Chlorzoxazone is a preferred probe for CYP2E1, so the pharmacokinetics of chlorzoxazone and turnover rate (TR) to 6-OH-chlorzoxazone, the principal metabolite of chlorzoxazone, in whole animals and in liver microsomes represents the activity of CYP2E1 in vivo and in vitro, respectively.

Pharmacokinetics of chlorzoxazone: Ten SD rats received an i.v. dose of 15 mg/kg chlorzoxazone and blood samples were collected at predose, 5, 15, 30, 60, 90 and 120 min by puncture of the orbital venous sinus. After a washout period of 4 days, the rats were established as the CIA model and the second pharmacokinetics study was conducted 34 days after the first immunization. The concentration of chlorzoxazone in plasma was determined by HPLC-UV [28].

Turnover rate (TR) of 6-OH-chlorzoxazone: Rat liver microsomes were prepared by differential centrifugation. The incubation mixture

contained rat liver microsomes, NADPH (1 mM), 100 mM phosphate buffer (pH 7.4) and chlorzoxazone. The activity of CYP2E1 is reported as TR of 6-OH-chlorzoxazone, the principal metabolite of chlorzoxazone [28].

Content of CYP2E1: The content of CYP2E1 in joint tissue and synovial tissue was determined by immunohistochemistry and immuno blot, respectively.

2.5. Effects of *Cyp2e1* knockout on CIA rats

Six- to eight-week-old wild-type (WT, n = 10) and *Cyp2e1*-knockout (n = 10) male SD rats were used to establish the CIA model. The onset time of symptoms and scores of the 4 paws were recorded. Haematoxylin and eosin (H&E) staining was used for histological analysis of ankle joints.

2.6. Effects of CYP2E1 inhibitor Q11 on CIA rats

From 65 male SD rats, ten rats were randomly selected as a control group (Group I), and the other 55 rats were used to establish CIA model. The seventh day after the second immunization was recorded as day 1. The model was successfully established in 50 rats which were randomly divided into five groups of 10 as follows: Group II (model): i.g. with normal saline (0.2 ml per mouse). Group III (Celecoxib): i.g. with celecoxib (5 mg/kg) [29] at day 1, then administered once every 24 h. Group IV (Q11 18.75 mg/kg), Group V (Q11 37.5 mg/kg), Group VI (Q11 75 mg/kg): i.g. with 18.75 mg/kg, 37.5 mg/kg and 75 mg/kg Q11. The dosing schedule of Q11 was same as the celecoxib group.

The volume of the hind paw, thickness and score of 4 paws were determined on 1th, 2th, 4th, 6th, 8th, 12th, 16th and 20th day. Blood was collected on day 8 and all the rats were euthanized on the 20th day.

2.7. Effects of CYP2E1 inhibitor Q11 on adjuvant-induced arthritis (AIA) rats

According to Liu and Wang et al[30,31], briefly, rats were immunized with Complete Freund's adjuvant (CFA, 0.1 ml/rat) by intradermal injection into the right hind metatarsal footpad. Fifty SD rats were randomly divided into a model group (saline, 0.5 mL/kg, i.g.), celecoxib group (celecoxib, 5 mg/kg, i.g.), low dose (Q11, 6 mg/kg, i.g.), medium dose (Q11, 30 mg/kg, i.g.), and high dose of CYP2E1 inhibitor group (Q11, 150 mg/kg, i.g.). The drugs were administered once before the administration of CFA, and then administered once a day. The volume of the foot of the rats was measured. The rats were euthanized on the 10th day and the ankle joints of each rat were collected.

2.8. Biochemical analysis

The content of TNF- α , IL-1 β and IL-6 in sera were determined with ELISA kits obtained from Hangzhou MultiSciences (Lianke) Biotech Co., Ltd. The level of malondialdehyde (MDA) and GSH was determined in serum using a kit purchased from Nanjing Jiancheng Bioengineering institute.

2.9. Cell treatment schedules

MH7A cells were cultured to establish an inflammatory cell model in vitro as stimulated by IL-1 β (10 ng/mL [32]).

CCK-8 method was used to detect the effects of Q11 on MH7A cells viability. Cells were treated with different concentrations of Q11(1.25, 5, 20, 80, 100 and 200 μ M) for 24 h. A 20 μ L CCK-8 solution (5 mg/ml) was added to each well and incubated for 2 h. The absorbance of the solution was measured of 490 nm with a microplate reader (Bio Tek, USA) and results were expressed as the percentage of the control cells.

For the migration and invasion assay, MH7A cells were seeded in 6-well plates and all groups except the blank group were treated with IL-1 β

(10 ng/ml) and varying concentrations of Q11 (5, 20 and 80 μ M) for 12 h at 37 °C and 5 % CO₂. MH7A cells were suspended in 200 μ L serum-free medium at a concentration of 6 × 10⁴ cells/mL and plated in the upper wells of transwell chambers (Corning, USA) with (invasion) or without (migration) Matrigel (BD Bioscience, USA). The bottom wells contained 600 μ L DMEM medium with 10 % FBS. After 12 h of incubation, the noninvasive cells on the upper membrane surface were removed by wiping with the cotton swab. The upper chamber was fixed with methanol and stained with 0.1 % crystal violet. Cells that migrated or invaded were photographed under microscope and four random fields were imaged.

A DCFH-DA fluorescent probe (Sigma Aldrich) and microporous plate methods were used to detect the effects of Q11 on ROS and GSH. The effect of Q11 on Nrf2 nuclear translocation was observed by immunofluorescence staining. The expression of Nrf2 and HO-1 in the nucleus was detected by western blot.

2.10. Western blot analysis

Equal amounts of protein were separated by 10 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane. The PVDF membrane was blocked with 5 % skim milk in a Tris-buffered saline with 0.1 % Tween 20 buffer (TBST) and incubated with the corresponding primary antibodies (Anti-CYP2E1, Sigma Antibody, #40815; Anti-Nrf2, Sigma Antibody 29048; Anti-HO-1, abcam, ab68477, Anti- β -Actin, Servicebio, GB11001) overnight at 4 °C. After three washes with TBST, the membrane was incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies for 60 min. Finally, an enhanced chemiluminescence kit and Gel Imaging System (Tanon Science & Technology CO, Shanghai, China) were used to identify antibody-bound proteins. Protein levels were quantified using Image J software and normalized to the control.

2.11. Statistical analysis

All experimental data were statistically analyzed and plotted by SPSS 22.0 and Graphpad Prism 8.0 software. A Shapiro-Wilk test was used to determine whether the data followed normal distribution. The normal distribution data were analyzed by t test (two-group data) and one-way ANOVA (multi-group data). Correlation analysis was performed by a Pearson test. P<0.05 indicates statistical significance.

3. Results

3.1. Changes in CYP2E1 expression in joint synovial tissue of RA patients

The datasets (GSE206848 and GSE48780) encompass a total of 101 samples, consisting of 87 samples from rheumatoid arthritis synovium cases and 7 samples from normal synovium cases (7 cases of synovial tissue from patients with osteoarthritis were not counted in this paper).

Using a screening threshold of log-fold change (logFC) > 1.5 and a P-value < 0.05, 1215 up-regulated genes and 746 down-regulated genes were identified (Fig. 1A). Differential analysis on GSE206848 and GSE48780 found that the expression of CYP2E1 mRNA was higher in RA patients than that in controls, whereas the expression of Nrf2 decreased (Fig. 1B and 1C).

3.2. The effects of *Cyp2e1*-/- on the occurrence and development of CIA rats

WT rats and *Cyp2e1*-/- rats were used to establish CIA models. The appearances of paws are shown in Fig. 1D. The red and swelling paw of *Cyp2e1*-/- was relieved compared with WT rats. The incidence rate of CIA in *Cyp2e1*-/- rats (40 %) was significantly lower than that of WT rats (90 %), and the onset time of symptoms (7.0 ± 0 d) was significantly later

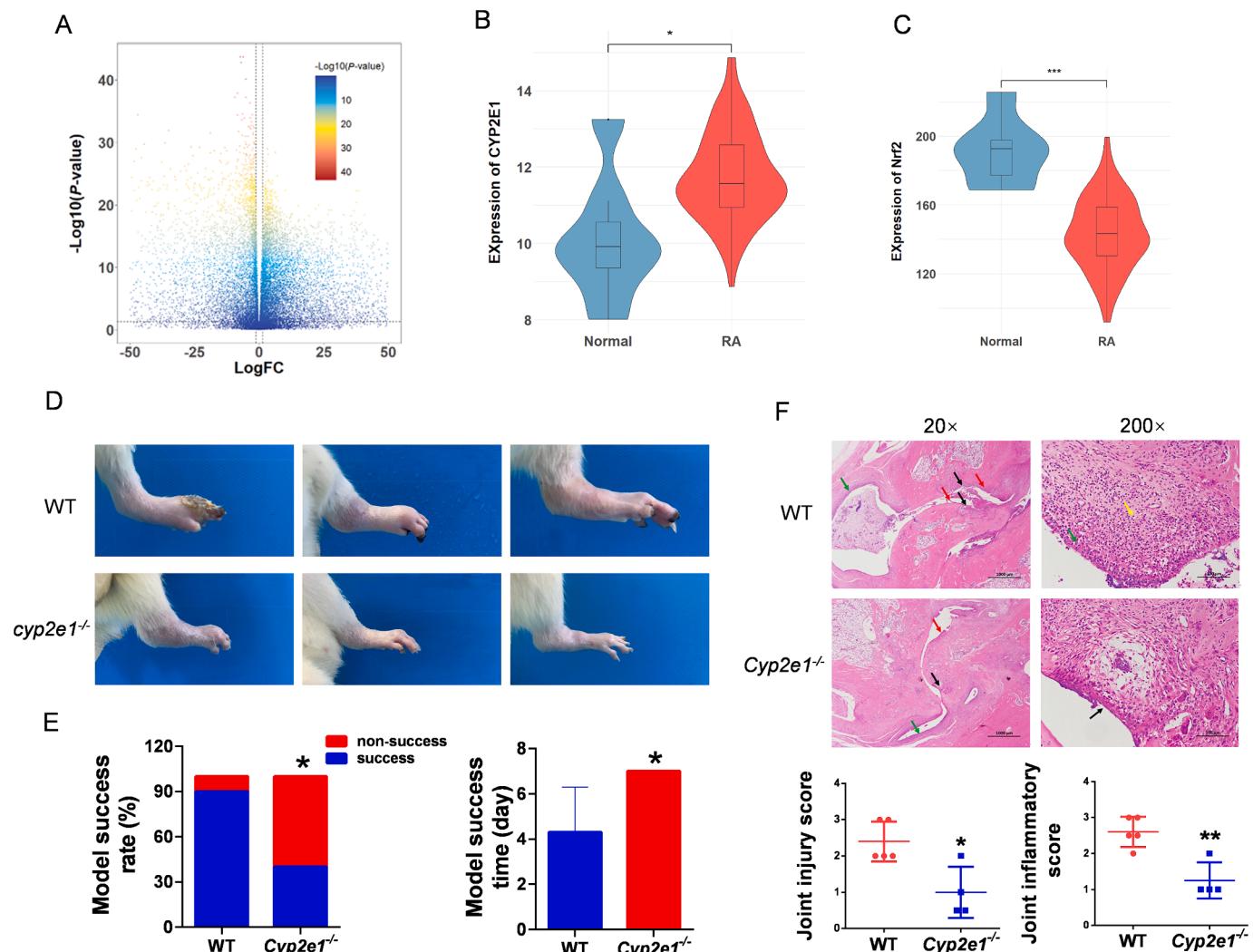


Fig. 1. CYP2E1 and Nrf2 expression in synovial tissue from gene-expression datasets and effects of *Cyp2e1*-/- on CIA rats (A) A volcano plot showing differentially expressed genes in RA patients compared to controls. (B-C) Expression of CYP2E1 and Nrf2 mRNA in synovial tissues of RA patients and normal human subjects. * $P<0.05$, *** $P<0.001$ vs. normal. (D) Pictures of paw swelling in each group (E) Effects of *Cyp2e1*-/- on occurrence and development in CIA rats (F) Effects of *Cyp2e1*-/- on pathological damage of ankle joint in CIA rats based on H&E staining in each group. Black arrow: cartilage damage; green arrow: connective and synovial tissue proliferation; yellow arrow: neutrophil infiltration; red arrow: pannus erosion. * $P<0.05$ and ** $P<0.01$ vs. WT.

than that of WT rats (4.3 ± 2.0 d) ($P<0.05$, Fig. 1E).

H&E staining was performed to observe synovial hyperplasia in ankle joints. Five rats of the WT group and four rats of the *Cyp2e1*-/- group were randomly selected for H&E staining. Compared to the WT group, the *Cyp2e1*-/- group showed less joint inflammation (synovitis), pannus formation and bone erosions (Fig. 1F). H&E staining was scored under the optical microscope and the results indicated that the ankle joint injury scores and inflammation scores in *Cyp2e1*-/- group were significantly lower ($P<0.05$, Fig. 1F).

The above results indicated that *Cyp2e1*-/- significantly decreased pathological injury and CYP2E1 could be used as a target for RA.

3.3. Changes in activity and expression of CYP2E1 in CIA rats

3.3.1. Change in CYP2E1 activity *in vivo*

The pharmacokinetics of chlorzoxazone, a probe of CYP2E1, were determined by a self-controlled study. The results show that there were significant changes in the pharmacokinetics of chlorzoxazone in rats before and after treatment with collagen (Fig. 2A-1). The clearance rate (CL) of chlorzoxazone (0.87 ± 0.13 (L/h/kg) was significantly higher than that before modeling (0.65 ± 0.08 (L/h/kg)) ($P<0.05$). The

concentration at 5 min ($C_{5\text{min}}$) and area under the curve (AUC) decreased 29.5 % and 28.0 % compared with that before modeling, respectively ($P<0.05$, Fig. 2A-2). The above results suggest that the activity of CYP2E1 increased significantly in CIA rats ($P<0.05$).

3.3.2. Change in CYP2E1 activity *in vitro*

The content of 6-OH-chlorzoxazone, a metabolite of chlorzoxazone, was measured by HPLC-UV to evaluate the changes in CYP2E1 activity in liver microsomes. Compared with the control, the activity of CYP2E1 in the model group was significantly increased ($P<0.05$, Fig. 2B-1), and there was a positive correlation between the activity and the paw swelling index (paw scores, thickness and volume) ($0.6 < r \leq 0.7$, $P<0.05$, Fig. 2B-2). The results suggest that the greater the activity of CYP2E1, the more severe the symptoms of RA.

3.3.3. Changes in CYP2E1 expression in synovial tissue

The expression of CYP2E1 in joint tissue and synovial tissue of normal rats and CIA rats was analyzed by immunohistochemistry (Fig. 2C) and western blotting (Fig. 2D), respectively. Compared with normal rat, CYP2E1 expression in CIA rats was significantly increased ($P<0.05$).

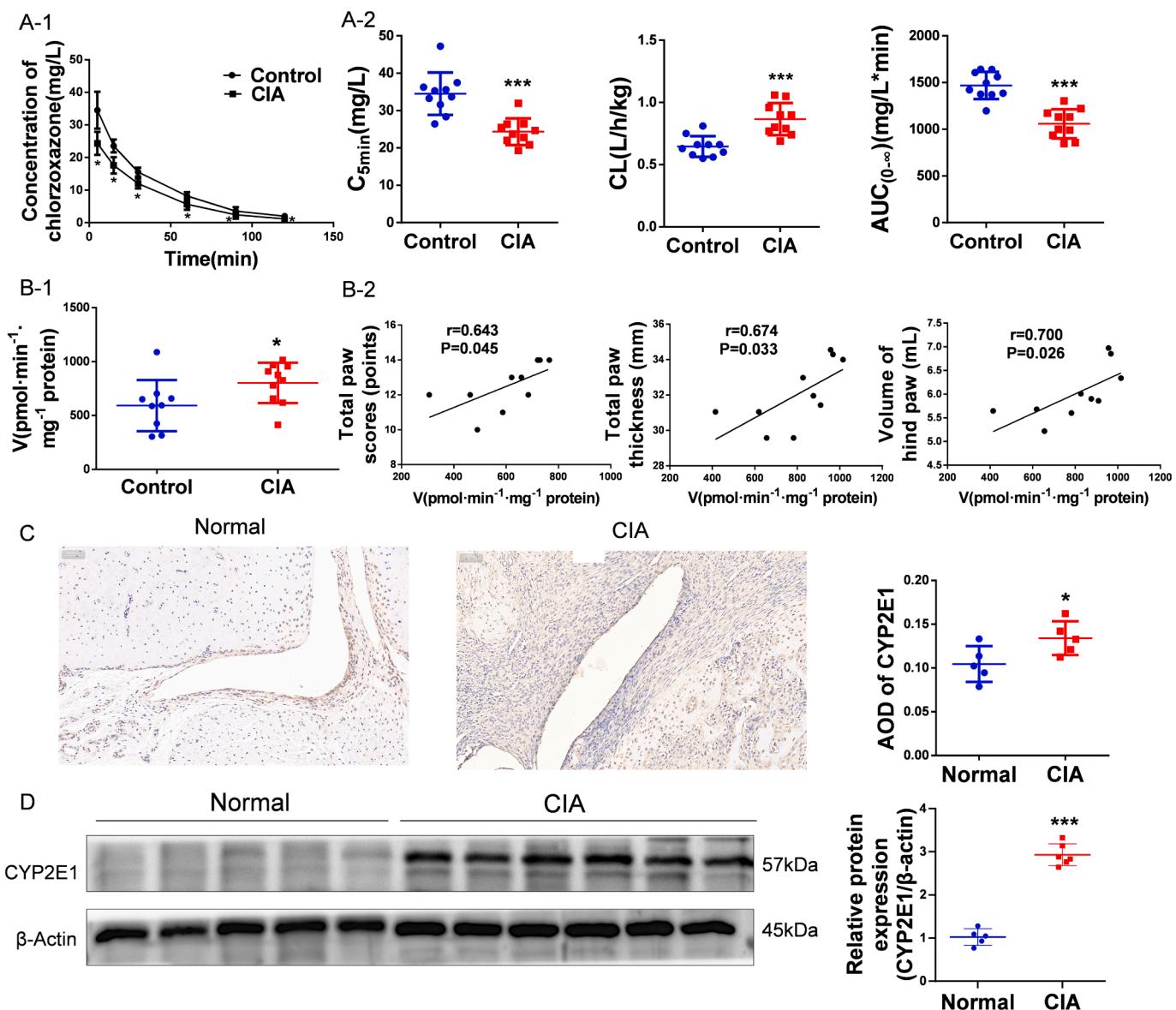


Fig. 2. Changes in CYP2E1 in vitro and in vivo in CIA rats (A-1) Plasma concentration of chlorzoxazone (15 mg/kg, i.v.). (A-2) Pharmacokinetic parameters of chlorzoxazone. (B) CYP2E1 activity in rat liver microsomes (B-1) and correlation with paw swelling (B-2). (C) Expression of CYP2E1 in ankle joint detected by IHC ($n = 5$, magnification of objective lens $20 \times$). (D) Expression of CYP2E1 in synovial tissues detected by western blotting. The protein bands were scanned quantitatively using the ImageJ. * $P < 0.05$, ** $P < 0.001$ vs. normal.

3.4. Effects of the CYP2E1 inhibitor Q11 on CIA rats

3.4.1. Effects of Q11 on paw swelling in CIA rats

The rats showed symptoms of RA successively from 3 to 7 days after the second immunization, and the 7th day after the second immunization was used as the first day of measurement and administration of Q11. From the 6th day of administration, Q11 significantly alleviated paw swelling-related indicators (paw scores, thickness and volume) ($P < 0.05$, Fig. 3A). Calculated by the total paw thickness, the highest recovery rate in celecoxib group, Q11 low, medium and high dose groups were ($34.0 \pm 19.4\%$), ($18.8 \pm 14.5\%$), ($32.2 \pm 19.9\%$) and ($43.9 \pm 23.3\%$), respectively (Fig. 3B-1). In conclusion, the relief of paw swelling at a medium dose of Q11 was similar to celecoxib and a high dose of Q11 was better than celecoxib on CIA rats. Moreover, the results indicate that the effects of Q11 on CIA rats were dose-dependent ($0.3 < r < 0.7$, $P < 0.05$, Fig. 3B-2).

H&E staining was scored under the optical microscope. Compared with the model group, the Q11 medium and high dose groups and the

celecoxib group showed less joint inflammation (synovitis), pannus formation, cartilage destruction and bone erosions (Fig. 3C-1). The ankle joint injury scores and inflammation scores in Q11 medium and high dose groups were significantly decreased ($P < 0.05$, Fig. 3C-2, C-3). Q11 can significantly improve the pathological injury of joints in CIA rats.

3.4.2. Effects of the CYP2E1 inhibitor Q11 on inflammatory and oxidative stress in CIA rats

Serum was collected at the peak of swelling at the 8th day and the level of inflammatory cytokines TNF- α , IL-1 β and IL-6 were measured by ELISA. The results show that Q11 significantly decreased the levels of TNF- α , IL-1 β and IL-6 in serum of CIA rats ($P < 0.05$, Fig. 4A).

The oxidative stress index MDA and antioxidant stress index GSH were determined, which indicated that Q11 significantly reduced the expression of MDA and increased the expression of GSH ($P < 0.05$, Fig. 4B) in CIA rats. The results suggest that the effects of Q11 on RA are related to oxidative stress.

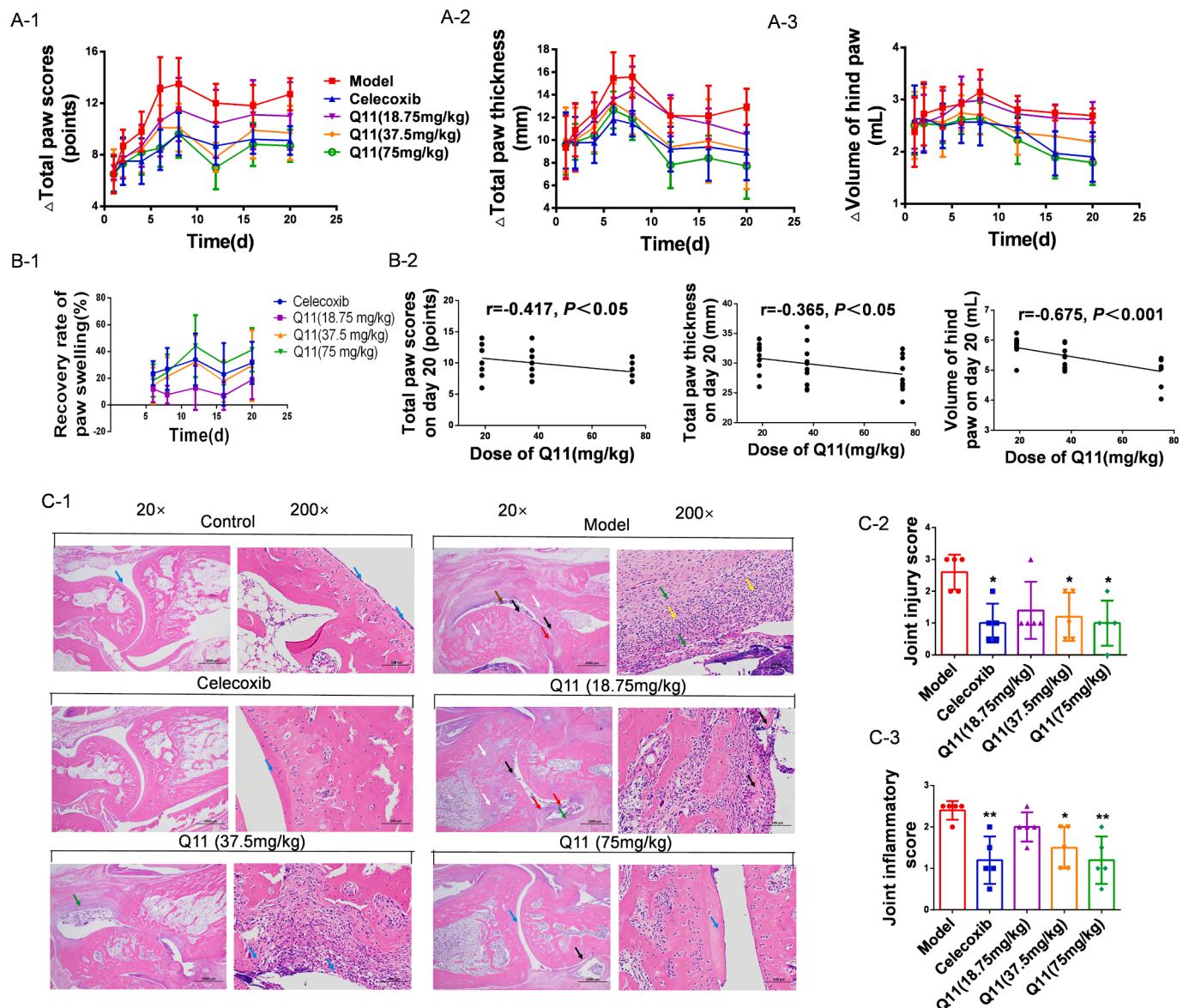


Fig. 3. Effects of CYP2E1 inhibitor Q11 on CIA rats. (A) Effects of Q11 on the dynamic change process of Δ total paw scores (A-1), Δ paw thickness (A-2) and Δ volume of hind paw (A-3) in CIA rats. (B-1) Recovery rate of Q11 on paw swelling in CIA rats. (B-2) Dose-effect relationship of Q11 to the paw swelling indexes in CIA rats. (C-1) Representative photograph of H&E staining of ankle joint sections. Ankle joint injury scores (C-2) and inflammatory scores (C-3) based on H&E staining in each group. * $P<0.05$, ** $P<0.01$ vs. Model group.

The joints of the rats were collected on the 20th day. Expression of HO-1 in joint tissues was detected by western blotting. We found that Q11 significantly increased HO-1 protein expression in joint tissue in CIA rats ($P<0.05$, Fig. 4C).

3.5. The effects of the CYP2E1 inhibitor Q11 on AIA rats

AIA rats were used to further confirm the effects of Q11. Compared with the model group, Q11 significantly inhibited paw swelling at 36 h ($P<0.05$, Fig. 5A-1), and the effect was maintained until the end of the experiment. The recovery rate of Q11 (30 mg/kg and 150 mg/kg) was similar to that of celecoxib on AIA rats (Fig. 5A-2). Moreover, the recovery rate with Q11 was significantly correlated with the dose ($0.4 < r < 0.7, P<0.05$, Fig. 5B).

The results of H&E staining showed that the synovial tissue of the ankle joint had proliferated significantly in the model group, while the proliferation of synovial tissues was not obvious in the CYP2E1 inhibition group (Fig. 5C-1). Moreover, the expression level of TNF- α in the

synovial epithelial cells of the ankle joint in the model group was significantly higher than that of the control group, while Q11 could reverse this change ($P<0.05$, Fig. 5C-2). The expression level of Nrf2 at 36 h after treatment with CFA in the synovial tissue in model group was significantly lower than that in the control group ($P<0.05$, Fig. 5C-3), which was consistent with the results of datasets related to rheumatoid arthritis (Fig. 1C). Moreover, the level of Nrf2 increased significantly after treatment with Q11 ($P<0.05$, Fig. 5C-3).

3.6. Mechanism of inhibition of Q11 on RA

3.6.1. Effects of Q11 on the function of MH7A cells

Synovial fibroblasts are the main effector cells in RA synovial tissue, with abnormal proliferation, migration and invasion, playing an important role in the pathophysiological process of RA. Therefore, MH7A cells were cultured to establish inflammatory cell models in vitro stimulated by IL-1 β .

Compared with the control group, proliferation, migration, and

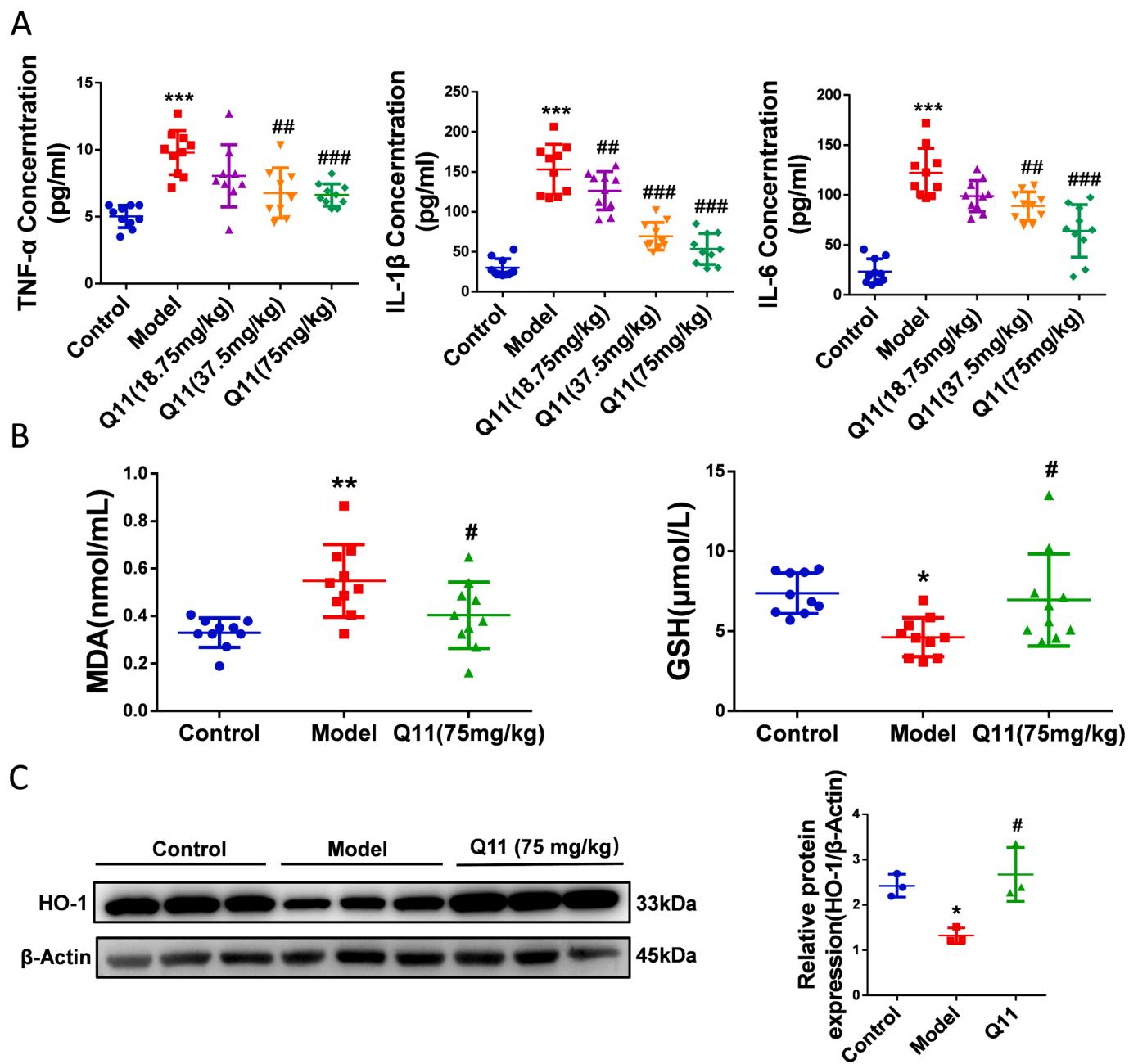


Fig. 4. The effects of Q11 on inflammatory and oxidative stress in CIA rats (A) Effects of Q11 on inflammatory cytokine levels in serum on peak paw swelling in CIA rats. (B) Effects of Q11 on oxidative stress indicator in serum on peak paw swelling in CIA rats. (C) Effects of Q11 on expression of HO-1 in CIA rats. * $P<0.05$ vs. Control; ** $P<0.01$ and *** $P<0.001$ vs. Model.

invasion MH7A cells increased significantly after 12 h treatment with 10 ng/mL IL-1 β ($P<0.05$). Compared with the IL-1 β group, the number of migrated and invasive cells decreased significantly after 12 h treatment with 20 μ M and 80 μ M of Q11 ($P<0.05$), and the number of proliferated cells decreased significantly after treatment with 100 μ M and 200 μ M of Q11 ($P<0.05$, Fig. 6 A, C, D). These results suggest that Q11 can inhibit proliferation, migration and invasion ability of MH7A cells.

3.6.2. The effects Q11 on the Nrf2/HO-1 pathway

A DCFH-DA fluorescent probe and microporous plate methods were used to determine the effects of Q11 on ROS and GSH in MH7A cells. The effects of Q11 on Nrf2 nuclear translocation was observed by immunofluorescence staining. The expression of Nrf2 and HO-1 in the nucleus was measured by western blotting in MH7A cells.

The results show that Q11 significantly reduced the ROS level ($P<0.05$) and increased the level of GSH (Fig. 6B, $P<0.05$) in MH7A cells induced by IL-1 β . Moreover, Q11 significantly enhanced Nrf2 nuclear translocation and significantly increased the expression of Nrf2 and HO-1 in the cell nucleus (Fig. 6E and F, $P<0.05$). Therefore, we speculate that the anti-rheumatoid arthritis effect of the CYP2E1 inhibitor Q11 may be related to the ROS/Nrf2/HO-1 pathway.

4. Discussion

The prevalence of RA is about 0.5 to 1.0 % and the peak age of onset is between 30 and 50 years old [33]. Though current therapy, using conventional DMARDs including methotrexate and leflunomide have proven to be effective, an insufficient-responder population and certain adverse events suggest exploring novel targets and drugs [34]. In the

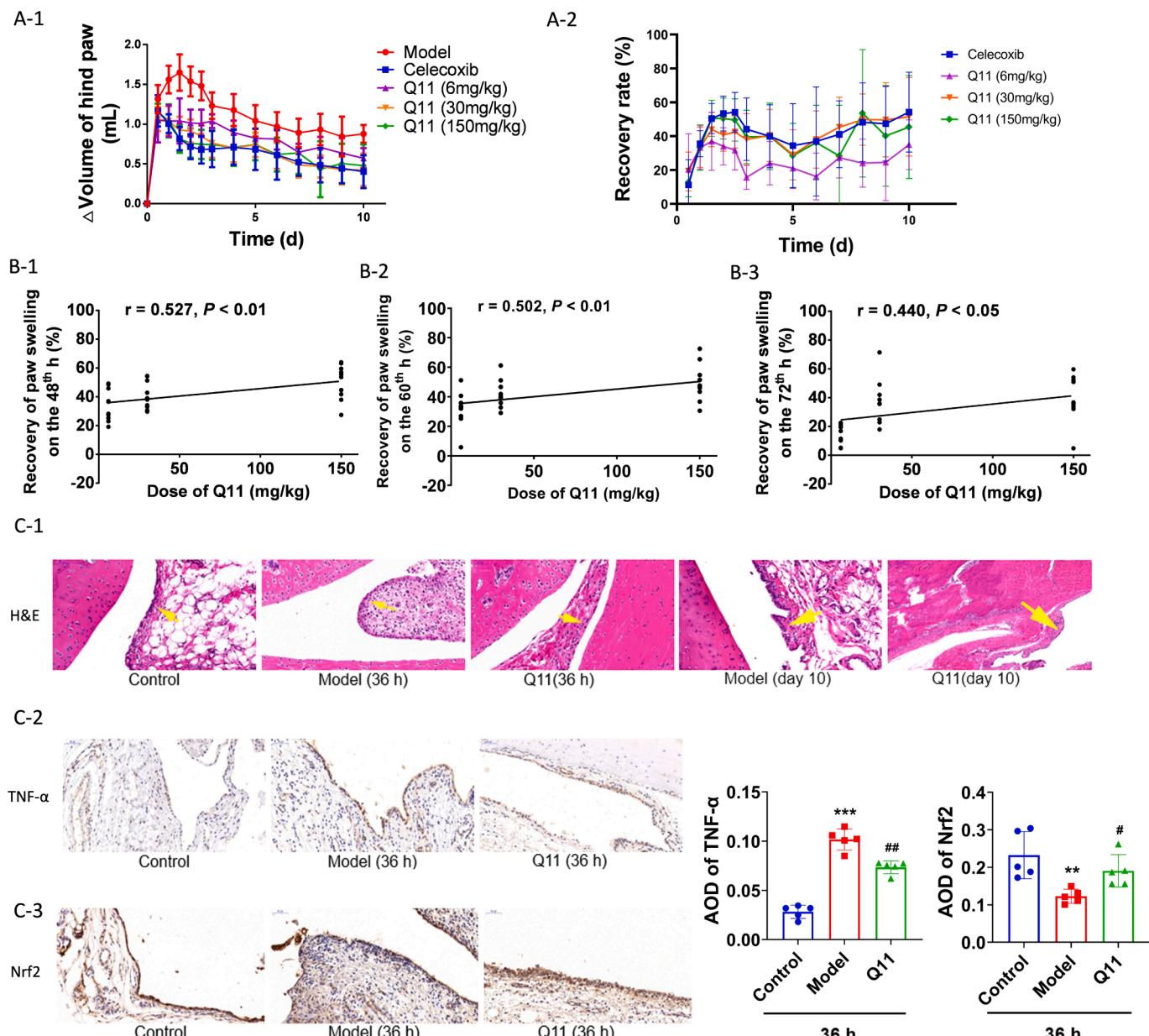


Fig. 5. The effects of the CYP2E1 inhibitor Q11 on AIA rats. (A-1) Change of volume of hind paw of AIA rats in model, positive control (celecoxib), and CYP2E1 inhibition (Q11) groups. (A-2) The recovery rate of Q11 at different time. (B) Dose-effect relationship of Q11 to the recovery of paw swelling on the 48 h, 60 h and 72 h in the AIA rat model. Representative image of H&E (C-1) and immunohistochemistry of TNF- α (C-2) and Nrf-2 (C-3) of synovial tissues of joints in AIA rats (magnification of objective lens 20 \times).

present study we found that the expression of CYP2E1 was elevated in synovial tissue of RA patients. We established a CIA model in *Cyp2e1*-/- rats and found that CYP2E1 might be a new target for RA treatment. Moreover, our results indicate that a new specific CYP2E1 inhibitor, Q11, has protective effects in CIA and AIA rats by decreasing oxidative stress and promoting an anti-inflammatory response via the ROS/Nrf2/HO-1 signaling pathways.

Both collagen-induced arthritis and adjuvant-induced arthritis can be used for RA research. Adjuvant-induced arthritis (AIA) in this paper focuses on acute inflammation; collagen-induced arthritis (CIA) is T helper cell-mediated, in which both T helper (Th1) and (Th17) responses are induced in CIA. The pathological and arthritic manifestations of the CIA model are more closely resemble human rheumatoid arthritis and the CIA model is the most used RA animal model [35]. There is no RA animal model which can mimic the human condition completely, so two types of RA animal models were used in this paper.

Fibroblast-like synoviocytes (FLS) play important role in the pathogenesis of RA with joint destruction [36]. Under physiological conditions the function of FLS is to build the lining layer of the synovium, secrete synovial fluid, and provide plasma protein for the adjacent cartilage and joint cavity [37]. In pathological synovial tissue in RA, FLS have the characteristics of tumor cells, which results in aggressive and invasive behavior of FLS in the adjacent cartilage and bone [36]. Therefore MH7A were chosen to construct an in vitro inflammatory model of RA stimulated by IL-1 β . Increased proliferation might contribute to the increased numbers of FLS and chronic inflammatory cells in RA joints [38]. Invasion of the synovium into the adjacent bone and cartilage is one of the characteristics of RA. The characteristic of destructive arthritis spreading between joints is mediated, at least in part, by the migration of activated FLS [39]. In this study, we found that Q11 significantly inhibited the proliferation, migration and invasive ability of MH7A cells. The results of HE also indicated that synovial

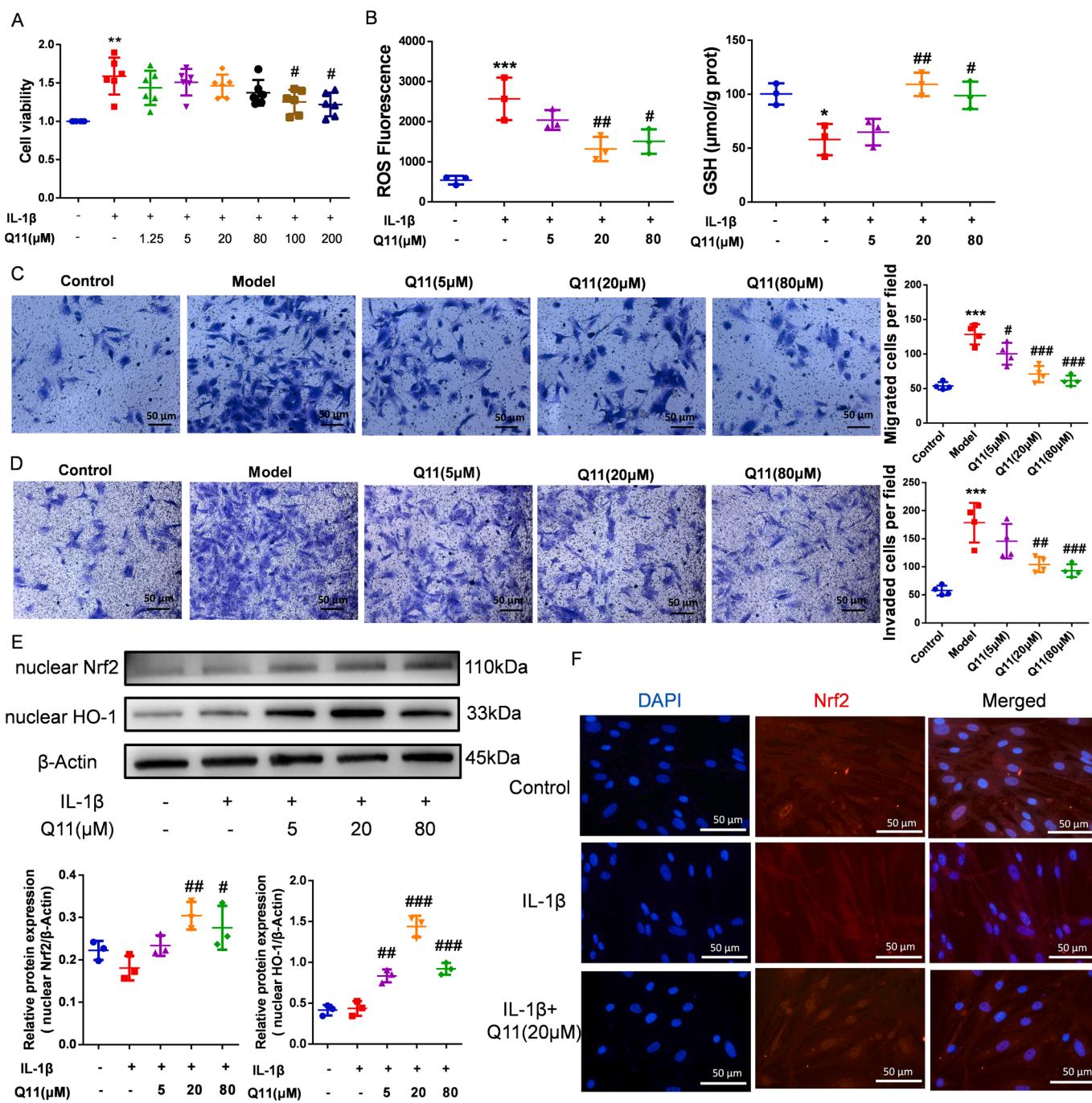


Fig. 6. The effects of Q11 on proliferation, migration and invasion and oxidative stress signaling pathway of MH7A cells induced by IL-1 β . (A, D) Effects of Q11 on proliferation, migration and invasion of MH7A cell. (B) Levels of ROS and GSH. (E) Nuclear levels of Nrf2 and HO-1 ($n = 3$). (F) Nrf2 nuclear translocation was observed by immunofluorescence staining. Each column represents the mean \pm SD. ** $P < 0.001$ vs. control group; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. IL-1 β group. Cells were treated with IL-1 β (10 ng/mL) and Q11 (5, 20, 80 μ M).

tissue hyperplasia and cartilage and bone destruction were significantly reduced, neutrophil infiltration was weakened, and no obvious pannus was observed in the Q11 treatment group compared with the model group. Taken together, we conclude that the mechanism of the therapeutic effect of Q11 on RA is at least partially related to the effects on FLS function.

In this study, three doses of Q11 on CIA rats (18.75 mg/kg, 37.5 mg/kg and 75 mg/kg) were chosen and the recovery rate on RA was (18.8 \pm 14.5) %, (32.2 \pm 19.9) % and (43.9 \pm 23.3) %, which indicated that the anti-RA effect increased significantly with increasing dose. Chlorzoxazone is a preferred probe for CYP2E1 activity in vitro and in vivo, and

6-OH-chlorzoxazone is the main metabolite of chlorzoxazone. The metabolic activity of CYP2E1 is represented by the TR of 6-OH-chlorzoxazone. In the present study we found that the expression of CYP2E1 was higher in RA patients and rats. Moreover, there was a positive correlation between CYP2E1 activity and the symptoms of rheumatoid arthritis, which indicated that CYP2E1 may not only be the target of RA, but also that CYP2E1 in the liver may be parallel to the joint. The change CYP2E1 expression in the clinical data is from gene-expression datasets, which are described at mRNA level and seemed mild. However, the change in expression of CYP2E1 in rat models was more significant at the protein level. The main reasons might be that CYP2E1 was primarily regulated

at the post-transcriptional and post-translational levels [15]. Murray et al also found that levels of Cyp2e1 mRNA in livers of Mat1a KO mice were increased only by 50 %, whereas CYP2E1 protein levels were 4.6-fold over the WT controls. The results also suggested that the increased CYP2E1 expression lies at the protein level [40].

Inflammation is a component of the occurrence and progression of various diseases, and especially for RA. In this study, the release of proinflammatory cytokines such as TNF- α , IL-6 and IL-1 β increased in CIA rats, and inflammatory cytokines lead to an imbalance of cytokines and immune dysfunction, which further aggravates symptoms of RA. Q11 suppressed the production of various cytokines, which demonstrates that Q11 has anti-inflammatory activity. The nuclear factor erythroid 2-related factor 2 (Nrf2) has been known to contribute to anti-inflammation through resistance to oxidative stresses. Under normal condition, Nrf2 is commonly degraded in the cytoplasm through binding to Keap1, a substrate adaptor for E3 ubiquitin ligase, which inhibits the transcriptional activity of Nrf2 via ubiquitination and proteasomal degradation. In response to oxidative stress, Nrf2 dissociates from Keap1 binding and translocates into the nucleus to form a heterodimer with members of small Maf proteins. Nrf2 regulates target gene expression through the antioxidant response element (ARE), present on genes such as HO-1 and glutathione S-transferase (GSH) [41,42]. Recent studies have focused on the relationship between RA and Nrf2. Nrf2 knockdown increased the incidence of arthritis, and the inflammatory response was enhanced, accompanied with joint destruction in front paws [43]. Wruck et al also reported that Nrf2 knockout mice had more severe cartilage injuries and more oxidative damage [44]. Zhai et al found that salicin, a prodrug form of aspirin, inhibits RA progress in CIA-induced rats, which may be related with Nrf2-HO-1-ROS pathways in RA-FLSs [11]. Xie et al reported that Wutou decoction ameliorates rheumatoid arthritis by regulating the NF- κ B and Nrf2 pathways [45]. Moreover, Puppala et al showed that Nrf2 expression was significantly reduced in an RA model group compared to the control group, and perillyl alcohol, which attenuates rheumatoid arthritis, reversed this decrease [46]. To sum up, the Nrf2 pathway plays an important role in RA.

There are some reports focused on the relationship between CYP2E1 and Nrf2. Cederbaum et al reported that Nrf2 plays a key role in the adaptive response against increased oxidative stress caused by CYP2E1 in HepG2 cells [12]. Cyp2e1 knockdown reduced ROS generation and elevated the expression of nuclear Nrf2 in HG-induced H9c2 and HL-1 cells [47]. Emeka et al reported that agents that inhibit CYP2E1 expression might attenuate mitoxantrone-induced cardiotoxicity by increasing Nrf-2 expression [48]. Xu et al found that patchouli alcohol (PA) ameliorates ethanol-induced acute liver injury partly related to restoration of CYP2E1/ROS/Nrf2/HO-1-mediated oxidative stress [49]. In this paper, Q11 significantly increased the expression Nrf2 and HO-1 in joint tissue in RA rats. Similar to the results in vivo, Q11 promoted Nrf2 translocation into the nucleus and activated target genes, such as HO-1 and GSH in MH7A cells. Our results indicate that the level of ROS significantly decreased in MH7A cells after treatment with Q11. Moreover, an indicator of antioxidant stress significantly increased in CIA rats after treatment with Q11.

In conclusion, CYP2E1 may be a new target for RA and Q11 has potential protective effects against RA by decreasing oxidative stress and promoting an anti-inflammatory response via the ROS/Nrf2/HO-1 signal pathway.

CRediT authorship contribution statement

Zixinying Han: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Chenxu Liu:** Visualization, Methodology, Formal analysis. **Mingrui Li:** Investigation, Conceptualization. **Mengyan Deng:** Investigation. **Ying Ding:** Investigation. **Yunchao Li:** Investigation. **Meidan Huo:** Investigation. **Haiwei Xu:** Investigation, Resources. **Hailing Qiao:** Supervision, Resources. **Na Gao:** Writing – original draft, Supervision, Project administration, Methodology,

Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NO. 82173889, 82274008, 82073930 and 81872931).

References

- [1] J.J. Cush, Rheumatoid arthritis: early diagnosis and treatment, *Rheum. Dis. Clin. North Am.* 48 (2) (2022) 537–547.
- [2] A. Finckh, B. Gilbert, B. Hodkinson, S.C. Bae, R. Thomas, K.D. Deane, D. Alpizar-Rodriguez, K. Lauper, Global epidemiology of rheumatoid arthritis, *Nat. Rev. Rheumatol.* 18 (10) (2022) 591–602.
- [3] M.H. Smith, J.R. Berman, What is rheumatoid arthritis? *J. Am. Med. Assoc.* 327 (12) (2022) 1194.
- [4] C. Patrono, Cardiovascular effects of cyclooxygenase-2 inhibitors: a mechanistic and clinical perspective, *Br. J. Clin. Pharmacol.* 82 (4) (2016) 957–964.
- [5] L.I. Filippini, R. Vercellino, N.P. Marroni, R.M. Xavier, Redox signalling and the inflammatory response in rheumatoid arthritis, *Clin. Exp. Immunol.* 152 (3) (2008) 415–422.
- [6] C.M. Quiñonez-Flores, S.A. González-Chávez, D. Del Río Nájera, C. Pacheco-Tena, Oxidative stress relevance in the pathogenesis of the rheumatoid arthritis: a systematic review, *Biomed Res. Int.* (2016), 6097417.
- [7] Y. Zamudio-Cuevas, K. Martínez-Flores, G.A. Martínez-Nava, D. Clavijo-Cornejo, J. Fernández-Torres, R. Sanchez-Sánchez, Rheumatoid arthritis and oxidative stress, *Cell. Mol. Biol. (noisy-Le-Grand)* 68 (6) (2022) 174–184.
- [8] R. Miesel, M.P. Murphy, H. Kröger, Enhanced mitochondrial radical production in patients which rheumatoid arthritis correlates with elevated levels of tumor necrosis factor alpha in plasma, *Free Radic. Res.* 25 (2) (1996) 161–169.
- [9] A. Somogyi, K. Rosta, P. Puszta, Z. Tulassay, G. Nagy, Antioxidant measurements, *Physiol. Meas.* 28 (4) (2007) R41–R55.
- [10] B. Kalpacioglu, K. Senel, The interrelation of glutathione reductase, catalase, glutathione peroxidase, superoxide dismutase, and glucose-6-phosphate in the pathogenesis of rheumatoid arthritis, *Clin. Rheumatol.* 27 (2) (2008) 141–145.
- [11] K.F. Zhai, H. Duan, G.J. Khan, H. Xu, F.K. Han, W.G. Cao, G.Z. Gao, L.L. Shan, Z. J. Wei, Salicin from *Alangium chinense* ameliorates rheumatoid arthritis by modulating the Nrf2-HO-1-ROS pathways, *J. Agric. Food Chem.* 66 (24) (2018) 6073–6082.
- [12] A. Cederbaum, Nrf2 and antioxidant defense against CYP2E1 toxicity, *Expert Opin Drug Metab.* 5 (10) (2009) 1223–1244.
- [13] R.V. Priyadarsini, S. Nagini, Quercetin suppresses cytochrome P450 mediated ROS generation and NFKB activation to inhibit the development of 7,12-dimethylbenz [a]anthracene (DMBA) induced hamster buccal pouch carcinomas, *Free Radic. Res.* 46 (1) (2012) 41–49.
- [14] G. Wang, M. Wakamiya, J. Wang, G.A.S. Ansari, M.F. Khan, Cytochrome P450 2E1-deficient MRL+/+ mice are less susceptible to trichloroethene-mediated autoimmunity: Involvement of oxidative stress-responsive signaling pathways, *Free Radic. Biol. Med.* 143 (2019) 324–330.
- [15] R. Harjumäki, C.S. Pridgeon, M. Ingelman-Sundberg, CYP2E1 in alcoholic and non-alcoholic liver injury. Roles of ROS, reactive intermediates and lipid overload, *Int. J. Mol. Sci.* 22 (15) (2021) 8221.
- [16] T.M. Leung, N. Nieto, CYP2E1 and oxidant stress in alcoholic and non-alcoholic fatty liver disease, *J. Hepatol.* 58 (2) (2013) 395–398.
- [17] T. Diesinger, A. Lautwein, S. Bergler, D. Buckert, C. Renz, R. Dvorsky, V. Buko, S. Kirk, E. Schneider, F. Kuchenbauer, M. Kumar, C. Günes, F. Genze, B. Büchele, T. Simmet, M. Haslbeck, K. Masur, T. Barth, D. Müller-Enoch, T. Wirth, T. Haehner, A new CYP2E1 inhibitor, 12-imidazolyl-1-dodecanol, represents a potential treatment for hepatocellular carcinoma, *Can. J. Gastroenterol. Hepatol.* 2021 (2021) 8854432.
- [18] A.I. Cederbaum, CYP2E1 potentiates toxicity in obesity and after chronic ethanol treatment, *Drug Metab. Drug Interact.* 27 (3) (2012) 125–144.
- [19] J. Yu, H. Zhu, M.S. Kindy, S. Taheri, Cytochrome P450 CYP2E1 suppression ameliorates cerebral ischemia reperfusion injury, *Antioxidants (Basel)* 10 (1) (2021) 52.
- [20] J. Zhou, Q. Wen, S.F. Li, Y.F. Zhang, N. Gao, X. Tian, Y. Fang, J. Gao, M.Z. Cui, X. P. He, L.J. Jia, H. Jin, H.L. Qiao, Significant change of cytochrome P450s activities in patients with hepatocellular carcinoma, *Oncotarget* 7 (31) (2016) 50612–50623.
- [21] J. Gao, Z. Wang, G.J. Wang, H.X. Zhang, N. Gao, J. Wang, C.E. Wang, Z. Chang, Y. Fang, Y.F. Zhang, J. Zhou, H. Jin, H.L. Qiao, Higher CYP2E1 activity correlates

- with hepatocarcinogenesis induced by diethylnitrosamine, *J. Pharmacol. Exp. Ther.* 365 (2) (2018) 398–407.
- [22] J. Gao, G.J. Wang, Z. Wang, N. Gao, J. Li, Y.F. Zhang, J. Zhou, H.X. Zhang, Q. Wen, H. Jin, H.L. Qiao, High CYP2E1 activity correlates with hepatofibrogenesis induced by nitrosamines, *Oncotarget* 8 (68) (2017) 112199–112210.
- [23] N. Gao, J. Chen, Y. Li, Y. Ding, Z. Han, H. Xu, H. Qiao, The CYP2E1 inhibitor Q11 ameliorates LPS-induced sepsis in mice by suppressing oxidative stress and NLRP3 activation, *Biochem. Pharmacol.* 214 (2023) 115638.
- [24] G. Hu, Y. Fang, H. Xu, G. Wang, R. Yang, F. Gao, Q. Wei, Y. Gu, C. Zhang, J. Qiu, N. Gao, Q. Wen, H. Qiao, Identification of cytochrome P450 2E1 as a novel target in glioma and development of its inhibitor as an anti-tumor agent, *Adv. Sci. (Weinh)* 10 (23) (2023) e2301096.
- [25] H.J. Li, C.T. Zhang, H. Du, T. Xu, Q. Li, P. Wang, G. Fang, G. Fan, Chemical composition of bawei longzuan granule and its anti-arthritis activity on collagen-induced arthritis in rats by inhibiting inflammatory responses, *Chem. Biodivers.* 16 (9) (2019) e1900294.
- [26] D.D. Brand, K.A. Latham, E.F. Rosloniec, Collagen-induced arthritis, *Nat. Protoc.* 2 (5) (2007) 1269–1275.
- [27] R. Caire, E. Audoux, G. Courbon, E. Michaud, C. Petit, E. Dalix, M. Chafchafi, M. Thomas, A. Vanden-Bosche, L. Navarro, M.T. Linossier, S. Peyroche, A. Guignandon, L. Vico, S. Paul, H. Marotte, YAP/TAZ: key players for rheumatoid arthritis severity by driving fibroblast like synoviocytes phenotype and fibro-inflammatory response, *Front. Immunol.* 12 (2021) 791907.
- [28] N. Gao, D. Zou, H.L. Qiao, Concentration-dependent inhibitory effect of Baicalin on the plasma protein binding and metabolism of chlorzoxazone, a CYP2E1 probe substrate, in rats *in vitro* and *in vivo*, *PLoS One* 8 (1) (2013) e53038.
- [29] H.H. Arab, A.M. Gad, E.M. Fikry, A.H. Eid, Ellagic acid attenuates testicular disruption in rheumatoid arthritis via targeting inflammatory signals, oxidative perturbations and apoptosis, *Life Sci.* 239 (2019) 117012.
- [30] Y.L. Liu, H.M. Lin, R. Zou, J.C. Wu, R. Han, L.N. Raymond, P.F. Reid, Z.H. Qin, Suppression of complete Freund's adjuvant-induced adjuvant arthritis by cobra toxin, *Acta Pharmacol. Sin.* 30 (2) (2009) 219–227.
- [31] S. Wang, Y. Wang, X. Liu, L. Guan, L. Yu, X. Zhang, Anti-inflammatory and anti-arthritis effects of taraxasterol on adjuvant-induced arthritis in rats, *J. Ethnopharmacol.* 187 (2016) 42–48.
- [32] M. Su, D. Zhou, J. Huang, T. Yang, Q. Zhou, Y. Tan, Forsythiaside A exhibits anti-migration and anti-inflammation effects in rheumatoid arthritis *in vitro* model, *Int. J. Rheum. Dis.* 27 (1) (2024) e14976.
- [33] E.M. Gravallese, G.S. Firestein, Rheumatoid arthritis - common origins, divergent mechanisms, *N. Engl. J. Med.* 388 (6) (2023) 529–542.
- [34] P. Prasad, S. Verma, N.K. Surbhi, V. Ganguly, S.A.M. Chaturvedi, Rheumatoid arthritis: advances in treatment strategies, *Mol. Cell. Biochem.* 478 (1) (2023) 69–88.
- [35] N. Choudhary, L.K. Bhatt, K.S. Prabhavalkar, Experimental animal models for rheumatoid arthritis, *Immunopharmacol. Immunotoxicol.* 40 (3) (2018) 193–200.
- [36] L.C. Huber, O. Distler, I. Turner, R.E. Gay, S. Gay, T. Pap, Synovial fibroblasts: key players in rheumatoid arthritis, *Rheumatology (Oxford)* 45 (6) (2006) 669–675.
- [37] Q. Ding, W. Hu, R. Wang, Q. Yang, M. Zhu, M. Li, J. Cai, P. Rose, J. Mao, Y.Z. Zhu, Signaling pathways in rheumatoid arthritis: implications for targeted therapy, *Signal Transduct. Target. Ther.* 8 (1) (2023) 68.
- [38] R.M. Pope, Apoptosis as a therapeutic tool in rheumatoid arthritis, *Nat. Rev. Immunol.* 2 (7) (2002) 527–535.
- [39] S. Lefèvre, A. Knedla, C. Tennie, A. Kampmann, C. Wunrau, R. Dinser, A. Korb, E. M. Schnäker, I.H. Turner, P.D. Robbins, C.H. Evans, H. Stürz, J. Steinmeyer, S. Gay, J. Schömerich, T. Pap, U. Müller-Ladner, E. Neumann, Synovial fibroblasts spread rheumatoid arthritis to unaffected joints, *Nat. Med.* 15 (12) (2009) 1414–1420.
- [40] B. Murray, H. Peng, L. Barbier-Torres, A.E. Robinson, T.W.H. Li, W. Fan, M. L. Tomasi, R.A. Gottlieb, J. Van Eyk, Z. Lu, M.L. Martínez-Chantar, S. Liangpunsakul, N.J. Skill, J.M. Mato, S.C. Lu, Methionine adenosyltransferase α1 is targeted to the mitochondrial matrix and interacts with cytochrome P450 2E1 to lower its expression, *Hepatology* 70 (6) (2019) 2018–2034.
- [41] E.H. Kobayashi, T. Suzuki, R. Funayama, T. Nagashima, M. Hayashi, H. Sekine, N. Tanaka, T. Moriguchi, H. Motohashi, K. Nakayama, M. Yamamoto, Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription, *Nat. Commun.* 7 (2016) 11624.
- [42] S.M. Ahmed, L. Luo, A. Namani, X.J. Wang, X. Tang, Nrf2 signaling pathway: Pivotal roles in inflammation, *Biochim. Biophys. Acta Mol. basis Dis.* 1863 (2) (2017) 585–597.
- [43] N. Maicas, M.L. Ferrández, R. Brines, L. Ibáñez, A. Cuadrado, M.I. Koenders, W. B. van den Berg, M.J. Alcaraz, Deficiency of Nrf2 accelerates the effector phase of arthritis and aggravates joint disease, *Antioxid. Redox Signal.* 15 (4) (2011) 889–901.
- [44] C.J. Wruck, A. Fragoulis, A. Gurzynski, L.O. Brandenburg, Y.W. Kan, K. Chan, J. Hassenpflug, S. Freitag-Wolf, D. Varoga, S. Lippross, T. Pufe, Role of oxidative stress in rheumatoid arthritis: insights from the Nrf2-knockout mice, *Ann. Rheum. Dis.* 70 (5) (2011) 844–850.
- [45] Y. Xie, C.T. Mai, D.C. Zheng, Y.F. He, S.L. Feng, Y.Z. Li, C.X. Liu, H. Zhou, L. Liu, Wutou decoction ameliorates experimental rheumatoid arthritis via regulating NF-κB and Nrf 2: Integrating efficacy-oriented compatibility of traditional Chinese medicine, *Phytomedicine* 85 (2021) 153522.
- [46] E.R. Puppala, S. Jain, P. Saha, M. Rachamalla, S. Np, S.S. Yalamarthi, M. Abubakar, A. Chaudhary, D. Chamundeswari, M. Usn, J.K. Gangasani, V.G.M. Naidu, Perillyl alcohol attenuates rheumatoid arthritis via regulating TLR4/NF-κB and Keap1/Nrf2 signaling pathways: A comprehensive study on *in-vitro* and *in-vivo* experimental models, *Phytomedicine* 97 (2022) 153926.
- [47] J. Wang, H. Yang, C. Wang, C. Kan, Cyp2e1 knockdown attenuates high glucose-induced apoptosis and oxidative stress of cardiomyocytes by activating PI3K/Akt signaling, *Acta Diabetol.* 60 (9) (2023) 1219–1229.
- [48] P.M. Emeka, H.I.M. Ibrahim, I.A. Alhaider, M.A. Morsy, M.E. Mohamed, Subchronic administration of mitoxantrone and the influence of enzyme inhibitors on its induced cardiotoxicity in mice: role of NRF-2/CYP2E1, *Eur. Rev. Med. Pharmacol. Sci.* 25 (24) (2021) 7806–7822.
- [49] L. Xu, Q. Huang, X. Tan, Q. Zhao, J. Wu, H. Liao, W. Ai, Y. Liu, Z. Lai, L. Fu, Patchouli alcohol ameliorates acute liver injury via inhibiting oxidative stress and gut-origin LPS leakage in rats, *Int. Immunopharmacol.* 98 (2021) 107897.