Hydroxychloroquine ameliorates DSS-induced colitis by inhibiting M1 macrophage polarization through NF-κB/NLRP3 signaling pathway

jingyue jiang  
The First Affiliated Hospital of Nanjing Medical University

Di Wang  
The Affiliated Hospital of Xuzhou Medical University

Shu Wang  
The First Affiliated Hospital of Nanjing Medical University

Ziping Ye  
The First Affiliated Hospital of Nanjing Medical University

Xiaqiong Mao  
The First Affiliated Hospital of Nanjing Medical University

XiaoJing Zhao  
The First Affiliated Hospital of Nanjing Medical University

Hongjie Zhang (✉ hjzhang06@163.com)  
The First Affiliated Hospital of Nanjing Medical University

Research Article

Keywords: Inflammatory bowel disease, Hydroxychloroquine, Macrophage polarization, colitis

Posted Date: October 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2134082/v1

License: ☒ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background and Aims: Polarization of macrophages to the M1 or M2 phenotype is critical for the development and occurrence of inflammation and disease. An increased proportion of M1-type macrophages has been observed in inflammatory bowel disease (IBD). Macrophage-targeted hydroxychloroquine (HCQ) nanotherapeutics are highly effective treatments for autoimmune diseases. HCQ has been widely used in the treatment of autoimmune diseases because of its anti-inflammatory and immunomodulatory properties. We investigated the potential effect of HCQ on dextran sulphate sodium salt (DSS)-induced colitis as well as the underlying potential mechanism.

Methods: C57BL/6 mice were randomly divided into three groups: control, DSS, or DSS + HCQ. The mRNA expressions of cytokines in M1 macrophages were measured using quantitative RT-PCR. The proportion of F4/80+CD86+ (M1) macrophages was measured by flow cytometry. Bone marrow-derived macrophages (BMDMs) were isolated and stimulated with LPS/IFN-γ to induce M1 macrophages. The levels of NF-κB p65, phospho-p65, NLRP3, ASC, and caspase-1 in the macrophages were detected by western blotting.

Results: HCQ ameliorates DSS-induced colitis as evidenced by improved colon shortening, reduced disease activity index (DAI), and decreased histopathological scores. Furthermore, HCQ decreased the expressions of M1-related cytokines and significantly decreased the percentage of F4/80+CD86+ (M1) subsets in colonic lamina propria cells in DSS-induced colitis mice. Moreover, HCQ significantly repressed M1 polarisation. HCQ can decrease the phosphorylation of NF-κB and the expressions of NLRP3, ASC, and caspase-1 in M1 macrophages. Blocking the NF-κB signaling pathway downregulated NLRP3 inflammasomes, and reduced the number of F4/80+CD86+ M1 macrophages.

Conclusions: HCQ attenuated colonic inflammation by inhibiting M1 macrophage polarization through the NF-κB/ NLRP3signaling pathway.

1. Introduction

Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD), is a chronic, non-specific inflammatory gastrointestinal disorder characterised by constant relapse and remission [1]. Although the exact aetiology and pathogenesis remain unclear, intestinal immune dysregulation appears to be closely associated with the occurrence of the disease in genetically susceptible individuals [2, 3]. Multiple immunological aspects have been considered to greatly contribute to IBD pathogenesis, including impaired skewing of macrophages or dendritic cells (DCs) with tolerogenic capacity, imbalance of Th17/Treg cells, and accumulated infiltration of various inflammatory cells [4]. In response to various signals from the surrounding tissue microenvironment, macrophage can be polarised to classically activated (M1 or pro-inflammatory) and alternatively activated (M2 or anti-inflammatory) macrophage phenotypes [5, 6]. M1 macrophages are abundant in the lamina propria of the inflamed mucosa of IBD patients and aggravate intestinal inflammation by releasing pro-inflammatory cytokines,
including TNFα, IL-1β, IL-12, and IL-6[4, 7]. As a result, modulating macrophage polarisation has recently been speculated to be a potential target for therapeutic intervention in IBD.

Various factors including IRF5, STAT1, and NF-κB are closely linked to M1 development [8]. In the intestinal immune system, the NF-κB transcription factor is one of the main modulators of pro-inflammatory gene transcription after activation by lipopolysaccharide (LPS) [9]. NF-κB activation initiates transcription of microglial nod-like receptor protein 3 (NLRP3). Macrophage M1 polarisation required NLRP3 inflammasome activation [10]. The NLRP3 inflammasome, a complex composed of NLRP3, ASC, and caspase-1, has been confirmed to promote the production of pro-inflammatory cytokines [11].

Hydroxychloroquine (HCQ) is widely known as a classic antimalarial agent. HCQ has anti-inflammatory and immunomodulatory effects and has been approved for the treatment of systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and other inflammatory rheumatic diseases [12–15]. HCQ interferes with lysosomal acidification [16], blocks antigen presentation, and downregulates cytokine release [17–19]. HCQ treatment targeted macrophages to inhibit M1 polarization and promote M2 polarization, which relieved articular inflammation [20].

Based on the beneficial effects of HCQ in treating immune-associated diseases, we investigated the potential effect of HCQ on intestinal inflammation using a dextran sulphate sodium (DSS)-induced colitis model and its possible mechanism. Our findings demonstrated that HCQ attenuated DSS-induced colitis by inhibiting M1 polarisation through inhibition of the NF-κB/NLRP3 pathway, which may offer new insights into the immunomodulatory effect of HCQ in IBD.

2. Materials And Methods

2.1. Animals and treatments

Male C57BL/6 mice (6–8 weeks, 18–20 g) were obtained from the Animal Center of the Nanjing Medical University (Nanjing, China). Animals were randomly divided into three groups: the control (n = 6), DSS model (n = 6), and HCQ-treated group (n = 6). The mice in the DSS model group were administered 3.5% (w/v) DSS (MP Biomedicals, 36,000–50,000 kDa) in their drinking water for 7 days, followed by 2 days of untreated water administration. The mice in the HCQ-treated group were administered HCQ (60 mg/kg, dissolved in 200 µL deionized water, Sigma-Aldrich) by gavage during DSS treatment from day 0 to 9. The mice in the control group were provided untreated drinking water until the end of the experiment. The animals were monitored daily (weight change, faecal bleeding, and stool consistency) to evaluate the disease activity index (DAI). All mice were sacrificed on day 10, and the colons were quickly removed for measurement of colon length or for further experiments. All the mice were housed under specific pathogen-free (SPF) conditions. The ethics committee of Nanjing Medical University approved this study. All procedures were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The ethics committee of Nanjing Medical University approved this
study. All procedures were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

### 2.2. Histological evaluation

The colons were completely removed, and approximately 0.5–1-cm colon sections were excised and fixed in 4% paraformaldehyde for 24 h for subsequent paraffin embedding, sectioning, and staining with haematoxylin and eosin (H&E). The histological score was calculated as follows [21]:

<table>
<thead>
<tr>
<th>score</th>
<th>term used</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>normal tissues</td>
</tr>
<tr>
<td>1</td>
<td>mild inflammation in the mucosa with some infiltrating mononuclear cells</td>
</tr>
<tr>
<td>2</td>
<td>increased level of inflammation in the mucosa with more infiltrating cells, damaged crypt glands and epithelium, mucin depletion from goblet cells</td>
</tr>
<tr>
<td>3</td>
<td>extensive infiltrating cells in the mucosa and submucosa area, crypt abscesses present with increased mucin depletion and epithelial cell disruption</td>
</tr>
<tr>
<td>4</td>
<td>massive infiltrating cells in the tissue, complete loss of crypts</td>
</tr>
</tbody>
</table>

### 2.3. Cell culture and treatment

After euthanatizing 6–8-week-old C57BL/6J mice, both the tibia and femur were removed, and the bone marrow was flushed out under aseptic conditions. After centrifugation for 5 min at 300 g and discarding of the supernatants, 5–10 mL of red blood cell lysis buffer (Beyotime) was added to eliminate red blood cells. Cells were washed twice with PBS and seeded in 6-well plates in RPMI 1640 medium (Gibco) supplemented with 10% (v/v) foetal bovine serum (FBS, Gibco), 1% penicillin/streptomycin (P/S, Invitrogen), and 10 ng/mL mouse colony-stimulating factor (M-CSF, Genescript) at 37°C in a humidified 5% CO₂ atmosphere for 7 days. The medium was changed on day 3, 5, and 7. After 7-day culture, primary bone marrow-derived macrophages (BMDMs) were obtained. LPS (100 ng/mL, Sigma-Aldrich) and 20 ng/mL interferon-γ (IFN-γ, Genescript) were used to induce M1 polarisation. Cells were pretreated with 10 µM HCQ (Sigma-Aldrich) for 2 h and PDTC for 30 minutes (Beyotime) before LPS/IFN-γ stimulation in the HCQ-treated group and PDTC treated group in vitro.

### 2.4. Isolation of colonic lamina propria mononuclear cells (LPMCs)

The entire colon was removed, and the bowel was incised lengthwise and cut into 0.5 to 1-cm pieces. Tissue pieces were then incubated in pre-warmed EDTA-PBS buffer (containing 0.5 mM EDTA) at 37°C for 40 min under slow rotation. Tissue pieces were washed with PBS to remove epithelial cells, and the remaining tissues were digested with 2 µg/mL DNase (Sigma-Aldrich) and 0.5 mg/mL collagenase IV (Sigma-Aldrich) at 37°C for 25 min. Next, the supernatants were filtered through a 100-µm mesh. The cells were resuspended in 40% Percoll and carefully added to 75% Percoll. After centrifugation, the interface
containing the lamina propria cells was collected and washed before use for flow cytometry analysis and RNA extraction.

### 2.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from BMDMs and colon samples using TRizol reagent (Invitrogen). cDNA was synthesized from 1 µg of total RNA using a reverse transcription kit (Vazyme Biotech), following the manufacturer's instructions. Real-time PCR was carried out using SYBR Green QPCR Master Mix (TaKaRa), and the relative expression of mRNAs was normalised to that of GAPDH. All primers used were as follows.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>forward: AGGTCGGTGTGAACGGATTGG</td>
</tr>
<tr>
<td></td>
<td>reverse: TGTAGACCATGTAGTTGAGGTCA</td>
</tr>
<tr>
<td>IL-1β</td>
<td>forward: AAGGGGACATTAGGCAGCAC</td>
</tr>
<tr>
<td></td>
<td>reverse: ATGAAAGACCTCAGTGCGGG</td>
</tr>
<tr>
<td>IL-23</td>
<td>forward: CAGCAGCTCTCTCGGAATCTC</td>
</tr>
<tr>
<td></td>
<td>reverse: TGGATACGGGGCACATTATTTC</td>
</tr>
<tr>
<td>IL-12</td>
<td>forward: CAATCACGCTACCTCCTTTTT</td>
</tr>
<tr>
<td></td>
<td>reverse: CAGCAGTGCAGGAATAATGTTC</td>
</tr>
<tr>
<td>iNOS</td>
<td>forward: GTTTCAGCCCAACAATACAAGA</td>
</tr>
<tr>
<td></td>
<td>reverse: GTGGACGGGTCGATGTCAC</td>
</tr>
<tr>
<td>TNF-α</td>
<td>forward: ACGGCATGGATCTCAAAGAC</td>
</tr>
<tr>
<td></td>
<td>reverse: GTGGGTGAGGAGCACGTAGT</td>
</tr>
</tbody>
</table>

### 2.6. Flow cytometry analysis

BMDMs and LPMCs were stained with antibodies (mAbs) against specific surface markers to detect changes in the M1 subtype. Briefly, BMDMs were washed and incubated with FITC-conjugated anti-mouse F4/80 and PE-conjugated anti-mouse CD86 antibodies. Cells isolated from the lamina propria were stained with Percp/cy5.5-conjugated anti-mouse CD11b, FITC-conjugated anti-mouse CD45, APC-conjugated anti-mouse F4/80, and PE-conjugated anti-mouse CD86. All antibodies used were purchased from BioLegend. After incubation at 4°C for 30 min in the dark and washing twice with PBS, all samples were analysed by flow cytometry (FACSCalibur, BD Biosciences). All data were analysed using the FlowJo V10 software.

### 2.7. Western blot
The cell lysate buffer contained 1 mL RIPA (P0013B, Beyotime), 0.1 mM PMSF reagent (ST506, Beyotime), and a protease inhibitor cocktail (P1005, Beyotime). BMDMs were washed twice with PBS and resuspended in cell lysate buffer. After lysis on ice for 20 min, the BMDMs were scraped off with a scraper and centrifuged for 15 min at 13000 g. The supernatant was collected. The protein samples were loaded onto an SDS-polyacrylamide gel for electrophoresis and transferred to a PVDF membrane. After blocking with a blocking buffer (P0222, Beyotime), primary antibodies against NLRP3 (1:1000, WL02635, Wanleibio), caspase-1 (1:1000, ab207802, Abcam), ASC (1:1000, D2W8U, CST), phospho-NF-κB p65 (1:1000, 3039S, CST), NF-κB p65 (1:1000, 8242S, CST), phospho-STAT1 (1:1000, ab109461, Abcam), STAT1 (1:1000, ab109320, Abcam), and IRF5 (1:1000, TA507252S, Origene) were incubated at 4°C overnight. The secondary antibodies were incubated for 1 hours at room temperature. Then, the protein bands were detected with ECL luminescence reagent, and band intensity was analysed using ImageJ software.

2.8. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Differences between two groups were calculated using Student’s t-test, while one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test was performed to analyse the differences among groups. Data were analysed using GraphPad Prism (version 7.0; IBM SPSS Statistics v21.0). A P value of < 0.05 was regarded as statistically significant and is shown as: *P < 0.05; ** P < 0.01; *** P < 0.001.

3. Results

3.1 HCQ ameliorates DSS-induced colitis

We used a DSS-induced experimental colitis model to investigate the potential effect of HCQ on intestinal inflammation. As shown in Fig. 1A, mice exhibited obvious weight loss from day 4 after DSS exposure, with diarrhoea and faecal bleeding observed in succession. In contrast, mice treated with HCQ showed improvements in weight loss, stool consistency, and faecal bleeding. The disease activity index (DAI) significantly decreased after HCQ treatment (Fig. 1B). In addition, HCQ-treated mice displayed significantly improved colon shortening, measured on day 10 after DSS exposure (Fig. 1C). Moreover, histological examination according to H&E staining of colon sections revealed prominent damage to the epithelial layer, crypt destruction, and increased immune cell infiltration after administration of DSS, however, histological scores could be reduced by treatment with HCQ (Fig. 1D). These results indicate that HCQ attenuated the severity and progression of colitis in DSS-treated mice.

3.2 HCQ reduces M1 macrophage polarization in DSS-induced colitis mice

To explore the impact of HCQ on macrophages, we isolated lamina propria mononuclear cells (LPMCs). First, we extracted total RNA from colonic LPMCs and detected the gene expression of the M1 cytokine...
profile. The results showed that the levels of M1-related proinflammatory cytokines (including IL-1β, IL-12, TNF-α, iNOS, IL-23, and IL-6) were significantly enhanced in DSS-induced colitis mice, whereas HCQ treatment decreased the mRNA expressions of IL-1β, IL-12, TNF-α, iNOS, and IL-23 (Fig.2A). Next, we explored whether HCQ contributed to correcting the increased percentage of M1 macrophages. After isolating colonic LPMCs, we assessed the phenotypic changes in the macrophages by flow cytometric analysis. As shown in Figure 2B, there were increased proportions of M1 macrophages (F4/80+CD86+ gated on CD45+CD11b+ cells) in DSS-exposed colonic LPMCs. HCQ treatment reduced the percentage of M1 macrophages to nearly normal levels. We also tested the frequency of M1 macrophages (F4/80+CD86+ gated on CD45+CD11b+ cells) in mesenteric lymph nodes (MLNs). DSS administration augmented the percentages of M1 macrophages, but HCQ treatment did not affect the M1 macrophage proportions in MLN.

Taken together, these results indicate that HCQ treatment inhibits the shift toward the M1 phenotype by decreasing M1 frequency and gene expression of specific M1-associated mediators in LPMCs, which may contribute to the remission of DSS-induced colitis.

3.3 HCQ suppresses M1 macrophage polarization in vitro

To determine whether HCQ affects M1 polarisation in vitro, we isolated mouse BMDMs to investigate the effect of HCQ on M1 polarisation in vitro. As shown in Fig. 3A, BMDMs were successfully induced, and the purity of F4/80+ CD11b+ cells was more than 90%, as determined by flow cytometric analysis. In addition, after stimulation with LPS/IFN-γ for 24 h, M1 macrophages displayed marked changes in cell shape with a flat, round, and pancake-like appearance photographed by white light confocal microscopy (Fig. 3B). Then, BMDMs were stimulated with LPS/IFN-γ alone or with LPS/IFN-γ and HCQ together, and the percentages of M1 macrophages (F4/80+CD86+) and gene expression of M1-related mediators were detected by flow cytometry and RT-PCR, respectively. M1 macrophage percentages were strongly induced after LPS/IFN-γ stimulation, whereas HCQ partially decreased the percentage of the M1 subtype (Fig. 4C). Moreover, mRNA expression of M1-related cytokines, including IL-1β, IL-12, IL-23, TNF-α, and IL-6, was obviously upregulated in BMDMs induced by LPS/IFN-γ; however, HCQ treatment reduced the expression of specific cytokines (Fig. 4D). Collectively, our results indicate that HCQ can inhibit LPS/IFN-γ-induced M1 macrophage polarisation and the expression of M1 macrophage-related pro-inflammatory cytokines in vitro.

3.4 HCQ inhibits M1 macrophage polarization through the NF-κB/NLRP3 pathway

Signal transduction factors such as IRF5, STAT1, and NF-κB are closely linked to M1 macrophage polarisation to further investigate the underlying mechanism, we collected cell lysates for protein expressions after LPS/IFN-γ stimulation with or without HCQ treatment. The phospho-p65 (p-p65) level
was significantly increased in the LPS group, while it was markedly reduced in HCQ treatment group (Fig. 4A). Our data showed that HCQ had no effect on IRF5 and STAT1 signalling (Fig. 4A). Since NF-κB activates a variety of genes in the nucleus, including the main component of the NLRP3 inflammasome, we examined the expression of the NLRP3 inflammasome in BMDMs. Compared to the control group, the protein expression of NLRP3, ASC, and caspase-1 increased in the LPS/IFN-γ group and decreased in the HCQ treatment group (Fig. 4B).

Furthermore, we used PDTC (a specific inhibitor of the NF-κB signalling pathway) to verify the involvement of NF-κB signalling and NLRP3 inflammasomes in M1 macrophages. Western blot analysis showed that PDTC decreased the phosphorylation level of NF-κB p65 and the expression levels of NLRP3, ASC, and caspase-1 in M1 macrophages ($P < 0.01$) (Fig. 4C-D). Flow cytometry also demonstrated that PDTC significantly decreased the number of F4/80$^+$CD86$^+$ M1 macrophages (Fig. 4E). These data suggest that HCQ inhibits M1 macrophage polarisation via NF-κB/NLRP3 pathway.

**Discussion**

Hydroxychloroquine (HCQ) is widely used to treat systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), antiphospholipid syndrome (APS), and other inflammatory rheumatic diseases [22-24]. Previous studies have shown that HCQ can attenuate renal/liver ischemia/reperfusion (I/R) injury by reducing inflammatory cytokine production [25, 26]. Kanvinde et al. demonstrated that polymeric chloroquine (PCQ) has a protective effect on colitis induced by *Clostridium rodenticus*, which is manifested by suppression of selected cytokines and recovery of intestinal inflammation [27]. In this study, we used DSS-induced colitis to observe the effect of HCQ on colitis. We found that HCQ could reduce the frequency of bloody stools, disease activity index (DAI), colon length, and recovery from histological damage. These results suggested that HCQ attenuates DSS-induced colitis.

As known HCQ has anti-inflammatory effects, in this study, we first determined the levels of inflammatory cytokines (IL-6, IL-1β, TNF-α, IL-12, and IL-23) in DSS-induced colitis with or without HCQ treatment, which played an important role in the pathogenesis of IBD. We found that the mRNA expressions of these inflammatory cytokines remarkably increased in DSS-induced colitis, while HCQ treatment decreased the mRNA levels of these pro-inflammatory cytokines.

Immunocytes including lymphocytes, dendritic cells, and macrophages, have been considered as the major source of these pro-inflammatory cytokines [21]. Macrophages can be polarised into classically activated or pro-inflammatory (M1) and alternatively activated or anti-inflammatory (M2) macrophages under the stimulation of a variety of factors from the intestinal microenvironment. M1 macrophages are characterised by the production of pro-inflammatory cytokines including IL-1β, IL-12, IL-6, and TNF-α. M2 macrophages have anti-inflammatory capacity, mainly by releasing IL-10 and promoting the expansion and differentiation of regulatory T cells (Tregs) [8, 28]. As previously reported, lamina propria macrophages (LPMϕs) can be classified as LPMϕ1 and LPMϕ2, and LPMϕ1 aggravates DSS-induced colitis [29]. In addition, an increased abundance of pro-inflammatory CD11c-high macrophages, rather
than tolerogenic CD11c phenotypes, has been found in the inflamed colons of IBD patients [30]. The study from Lissner et al. proved a shift towards pro-inflammatory M1 subsets of lamina propria macrophages in the inflamed mucosa of IBD patients [4].

Based on HCQ attenuating DSS-induced colitis and decreasing inflammatory cytokines (IL-6, IL-1β, TNF-α, IL-12, and IL-23), we further explored that the protective effect of HCQ against colitis may be involved in macrophage polarization and production of pro-inflammatory cytokines, and found that HCQ treatment diminished the percentage of M1 subsets and simultaneously the expressions of specific M1-associated pro-inflammatory cytokines in isolated colon lamina propria cells compared to that in DSS-induced mice. In parallel, we cultured BMDMs and established an *in vitro* model of M1 macrophages by LPS/IFN-γ stimulation. Similar to the *in vivo* results, HCQ treatment effectively reduced the gene expressions of the primary M1 cytokine profile and the percentage of the M1(F4/80+CD86+) subtype. These results suggested that HCQ can inhibit M1 polarisation and production of M1-associated cytokines.

Currently, the mechanism by which HCQ reduces M1 polarisation has not been thoroughly studied. In this study, we found that HCQ treatment reduced the phosphorylation of NF-κB/p65 in M1 macrophages. However, the STAT1 and IRF5 signalling pathways were not influenced by HCQ. Moreover, HCQ treatment can reduce the expression levels of NLRP3, ASC, pro-caspase-1, caspase-1, and IL-1β. After treatment with the NF-κB inhibitor PDTC, the level of p-NF-κB/p65, NLRP3, caspase-1, pro-caspase-1, and IL-1β decreased in BMDMs compared with that in the LPS/IFN-γ group. The percentage of F4/80+CD86+ M1 macrophages was partially reduced after PDTC pre-treatment. These results suggest that HCQ reduces M1 polarisation by inhibiting the activation of the NF-κB and NLRP3 inflammasomes.

Collectively, our results demonstrated that HCQ inhibits M1 macrophage polarisation through inhibiting the activation of the NF-κB/NLRP3 signal pathway, which contributes to alleviating DSS-induced colitis. Thus, our study provides evidence that HCQ may be a potential therapeutic agent for IBD.

**Conclusion**

In conclusion, our results indicated that Hydroxychloroquine suppressed M1 macrophage polarization and thus ameliorates colonic damage induced by DSS. We provided new evidence that the inhibition of M1 macrophage polarization attributed to down-regulation of NF-κB pathway and NLRP3 inflammasomes, which might be a new target of IBD treatment in the future.

**Abbreviations**

IBD  Inflammatory bowel disease

HCQ  Hydroxychloroquine
Declarations

Ethics approval and consent to participate

All protocols were approved by the Nanjing University of Science and Technology Animal Care and Use Committee.

Consent for publication

Not applicable.

Availability of data and materials

The data used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this article.
Funding

This work was supported by the Grant from the National Natural Science Foundation of China [Grant Nos. 82070568].

Author contributions

Jingyue Jiang and Di Wang contributed to this research equally. Hongjie Zhang conceived and designed the experiments; Jingyue Jiang, Di Wang, Shu Wang and Ziping Ye performed the experiments; Xiaqiong Mao and XiaoJing Zhao analyzed the data; Jingyue Jiang and Hongjie Zhang wrote the paper. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References


**Figures**
HCQ administration ameliorates the severity of DSS-induced acute colitis in mice. (A) Murine DSS-induced colitis and HCQ treatment protocol. Mice were divided into 3 groups: Mice in control group (n=6) were given normal water throughout the experiment. Mice in DSS group (n=7) were administered with 3.5% (w/v) DSS for 7 days followed by 2 days of water. Mice in DSS+HCQ group were given 60 mg/kg HCQ by oral gavage daily during DSS administration till the end of the experiment; (B) Body weight change and DAI.
were monitored daily; (C) Representative macroscopic images of colons and colon lengths in each group. (D) Representative H&E staining of colon sections were shown and histological scores for each group were assessed. Scale bars: 50 μm; All data are expressed as mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001. HCQ, hydroxychloroquine; DSS, dextran sulfate sodium; DAI, disease activity index; H&E, hematoxylin and eosin.

Figure 2
HCQ reduces M1 infiltration and gene expression of M1-related pro-inflammatory mediators in colonic LPMCs following DSS exposure. (A) The expression of M1-related pro-inflammatory mediators were analysed by RT-PCR in colonic LPMCs (B) Percentages of M1 subtype in LPMCs of each group were tested using flow cytometry. LPMCs were isolated from freshly removed colon tissues, then were stained with CD45, CD11b, F4/80 and CD86, F4/80+CD86+ populations gated on CD45+CD11b+ cells were considered as M1 subtypes. Data are expressed as mean ± SEM. (n=6-7 per group). **p < 0.01; ***p < 0.001. HCQ, hydroxychloroquine; DSS, dextran sulfate sodium salt; LPMCs, lamina propria mononuclear cells; RT-PCR, real time-polymerase chain reaction.
Figure 3

HCQ reduces M1 polarization and production of pro-inflammatory mediators in vitro. BMDMs were generated with 10ng/ml M-CSF for 7 days. LPS (100ng/ml) and IFN-γ (20ng/ml) were used to polarize M1 macrophages. (A) The percentage of mature macrophages after 7 days of culture with 10ng/ml M-CSF using flow cytometry; (B) Morphologies of untreated BMDMs (left) or treated with LPS/IFN-γ (right); scale bars: 50μm. (C) The percentages of M1 subtype among BMDMs without treatment, BMDMs treated with...
LPS/IFN-γ alone or combined with HCQ pretreatment were tested using flow cytometry; F4/80+CD86+ cells were regarded as M1 subsets; (D) The expression of M1-associated cytokines after stimulation with LPS/IFN-γ alone or together with HCQ (10 μM) for 12h using RT-PCR. Data are representative of three independent experiments, *p < 0.05; **p < 0.01; ***p < 0.001. BMDM, bone marrow derived macrophages; M-CSF, mouse-colony stimulating factor.

Figure 4

HCQ suppresses the activation of NLRP3 inflammasome and NF-κB signal. (A) Western blot showed changes in p-p65 (p-NF-κB p65), STAT1, IRF5, (B) NLRP3, ASC, and caspase-1 production in macrophages after co-culture with LPS/IFN-γ for 24 hours with or without HCQ pretreatment. (C) Western blot showed changes in p-p65 (p-NF-κB p65), (D) NLRP3, ASC, and caspase-1 production in macrophages after co-culture with LPS/IFN-γ for 24 hours with or without PDTC pretreatment. (E) Flow cytometry showed that PDTC pretreatment significantly decreased the number of F4/80+CD86+ M1 macrophages. Data are representative of three independent experiments, *p < 0.05; **p < 0.01; ***p < 0.001.