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Original Research

MCC950 Suppresses Hepatic Inflammaging by Inhibiting NLRP3 Inflammasome Activation in Spontaneously Aged Mice

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Abstract

Background: Aging is frequently accompanied by chronic, low-grade inflammation, often referred to as "inflammaging", which contributes to functional decline of multiple organs including the liver. The NLRP3 inflammasome has emerged as a key mediator of age-related inflammation; however, its pharmacological inhibition in the context of hepatic aging remains insufficiently explored. In this study, we investigated the effects of the selective NLRP3 inflammasome inhibitor MCC950 on inflammatory responses in the liver of aged mice. **Methods**: Aged C57BL/6 mice (18 months old) were administered MCC950 intraperitoneally for four weeks, and liver tissues were analyzed for inflammatory and stress-related markers. **Results**: MCC950 treatment significantly reduced hepatic expression of NLRP3, caspase-1 activation, and IL-1β production, accompanied by a decrease in proinflammatory cytokines such as p-STAT3. Histological analysis demonstrated attenuation of age-associated hepatic inflammatory infiltration and improved tissue architecture. Furthermore, MCC950 administration restored autophagy-related proteins (LC3B, p62) indicating broader protective effects on liver homeostasis. **Conclusion**: These findings suggest that NLRP3 inflammasome inhibition with MCC950 alleviates age-associated hepatic inflammation and may represent a potential therapeutic strategy for mitigating inflammaging and preserving liver function in the elderly.

Keywords: inflammasomes; liver; aging; NLRP3 inflammasome inhibitor; autophagy

1. Introduction

Aging is accompanied by progressive structural and functional deterioration in various organs, leading to increased susceptibility to chronic diseases and reduced physiological resilience. Among these age-related changes, chronic, low-grade systemic inflammation—commonly termed "inflammaging"—has emerged as a major contributor to the onset and progression of aging-associated pathologies [1]. The liver, a central organ in metabolism, detoxification, and immune regulation, is particularly vulnerable to age-driven inflammatory and oxidative insults. Accumulating evidence suggests that age-related hepatic inflammation accelerates fibrosis, metabolic dysregulation, and diminished regenerative capacity, ultimately impairing liver homeostasis and functional reserve [2,3].

A key molecular driver of inflammaging is the nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain-containing protein 3 (NLRP3) inflammasome [4]. The NLRP3 inflammasome is a cytosolic multiprotein complex that detects cellular stress signals and activates caspase-1, resulting in the maturation and release of proinflammatory cytokines such as interleukin-1β (IL-1β) and IL-18. Activation of the NLRP3 inflammasome is known to be enhanced in aged tissues, contributing to chronic inflammatory signaling, mitochondrial dysfunction, and impaired autophagic clearance [5]. In the liver, dysregulated NLRP3 signaling has been implicated in age-associated hepatocellular injury, steatohepatitis, and fibrosis, highlighting its potential as a therapeutic target for alleviating hepatic inflammaging [6].

Despite its pathogenic significance, pharmacological interventions targeting NLRP3 in the context of hepatic aging remain limited. MCC950 is a selective and potent small-molecule inhibitor of the NLRP3 inflammasome that prevents inflammasome assembly and caspase-1 activation without affecting other inflammasome complexes [7]. Preclinical studies have demonstrated the therapeutic benefit of MCC950 in metabolic, autoimmune, and neuroinflammatory disorders [5]; however, its effects on age-associated liver inflammation and tissue homeostasis have not been fully elucidated.

Therefore, the present study aimed to evaluate whether NLRP3 inhibition by MCC950 can attenuate inflammaging-related changes in the liver of aged mice. We assessed inflammatory cytokine expression, inflammasome activation, and autophagy-related markers to determine the therapeutic potential of MCC950 in restoring hepatic function during aging. Our findings provide evidence

that MCC950 alleviates age-related hepatic inflammation and may serve as a promising strategy to preserve liver health in the elderly population.

2. Materials and Methods

2.1 Reagents and Antibodies

MCC950 was purchased from AppexBio (Houston, TX, USA). The following primary antibodies were used in this study: NLRP3 (NLR family pyrin domain containing 3, 68102-1-1g, Proteintech, Rosemont, IL, USA); ASC (apoptosis-associated speck-like protein containing a CARD, A1170); VPS34 (vacuolar protein sorting 34, A12483), and LC3B (microtubule-associated protein 1A/1B-light chain 3B, A19665, Abclonal, Woburn, MA, USA); caspase-1 (sc-392736) and IL (interleukin)-1β (sc-12742, Santa Cruz Biotechnology, Dallas, TX, USA); phospho-STAT3 (#4113) and p62 (#5114, Cell Signaling Technology, Danvers, MA, USA); α-SMA (smooth muscle actin, ab5694); TGF (transforming growth factor)-β1 (ab64715) and GAPDH (glyceraldehyde-3-phosphate dehydrogenase, ab8245, Abcam, Cambridge, UK); F4/80 (14-4801-82, Invitrogen, Carlsbad, CA, USA). Appropriate HRP-conjugated secondary antibodies (genDEPOT, Barker, TX, USA) were used for signal detection according to the host species of the primary antibodies. Primary antibodies were diluted 1:1000 in antibody dilution buffer and incubated with the membranes, followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies diluted 1:5000.

2.2 Experimental Animals

The animal experimental protocols were approved by the Institutional Animal Ethics Committee of Chungnam National University (approval number: 202307A-CNU-136). Two-months old C57BL/6J male mice and 18-month-old C57BL/6J mice were obtained from the Animal Facility of Aging Science, Korea Basic Science Institute Gwangju Center (Gwangju, Korea). The mice were acclimated for 1 week and fed a pelleted diet and tap water *ad libitum* under specific pathogen-free conditions. The mice were randomly divided into three groups (n = 6/group) as follows: 2-months old C57BL/6J male mice (Young mice), 18-months old C57BL/6J male mice (Aged mice), and 18-months old C57BL/6J male mice intraperitoneally injected 20 mg/kg of MCC950 every other day (once every 48 hours) for 4 weeks (Aged mice + MCC). The mice were fasted prior to autopsy. Mice were euthanized using CO_2, with compressed CO_2 introduced at a rate of 30–70% of the chamber volume per minute (gradual-fill method) using a calibrated flow meter, and cardiac blood was subsequently collected.

2.3 Histological Analysis

Liver tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned at 4 µm thickness. After serial gradient hydration, sections were stained with hematoxylin and eosin (H&E) and Sirius Red using standard protocols. For immunohistochemistry (IHC), antigen retrieval was performed by heating the sections in citrate buffer (pH 6.0), followed by incubation with an anti-NLRP3 antibody (Proteintech, 1:200 dilution) and anti-F4/80 (Invitrogen, 1:400 dilution) overnight at 4 °C. Signal was detected using a avidin-biotin complex kit and DAB chromogen (VectorLabs, Burlingame, CA, USA). Each stained section (ten randomly selected fields) was photographed at 200× magnification using a light microscope (Olympus BX53, Tokyo, Japan). Inflammatory cell infiltration was assessed on H&E-stained liver sections, where inflammatory cells were identified by nuclear morphology and manually counted in five high-power fields per mouse. The average number of cells per field was used for analysis. Fibrosis was evaluated by quantifying the Sirius Red–positive area using ImageJ software (NIH, Bethesda, MD, USA). Collagen-stained regions were segmented using a fixed color threshold, and the fibrotic area was expressed as a percentage of total tissue area. NLRP3-positive cells and F4/80-positive cells were quantified by measuring the immunopositive area per field using the same imaging setup.

2.4 Measurement of Serum AST and ALT

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using commercial assay kits (ALT: AM103-K; AST: AM102; Assan Pharmaceutical, Seoul, Republic of Korea) according to the manufacturer's instructions. Briefly, the substrate reagent was pre-incubated at 37 °C, serum was added, and the reaction was allowed to proceed at 37 °C (ALT: 30 min; AST: 60 min). After color development with 2,4-dinitrophenylhydrazine followed by alkalinization, absorbance was measured at 505 nm (acceptable range: 490–530 nm) using a microplate reader, and enzyme activities were calculated from the manufacturer-provided standard curve and expressed as units per liter (U/L). All samples were assayed in triplicate to ensure analytical reproducibility.

2.5 Western Blot Analysis

Liver tissues were homogenized in radioimmunoprecipitation assay (RIPA) buffer (Cell Signaling Technology, Danvers, MA, USA) containing protease and phosphatase inhibitors and clarified by centrifugation. Protein concentrations were determined prior to analysis. Equal amounts of protein were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred using a wet transfer system (Bio-Rad, Hercules, CA, USA) onto polyvinylidene fluoride membranes (Millipore, Boston, MA, USA). Following transfer, membranes were blocked to minimize nonspecific binding and subsequently incubated with the appropriate primary antibodies under controlled conditions. After wash-

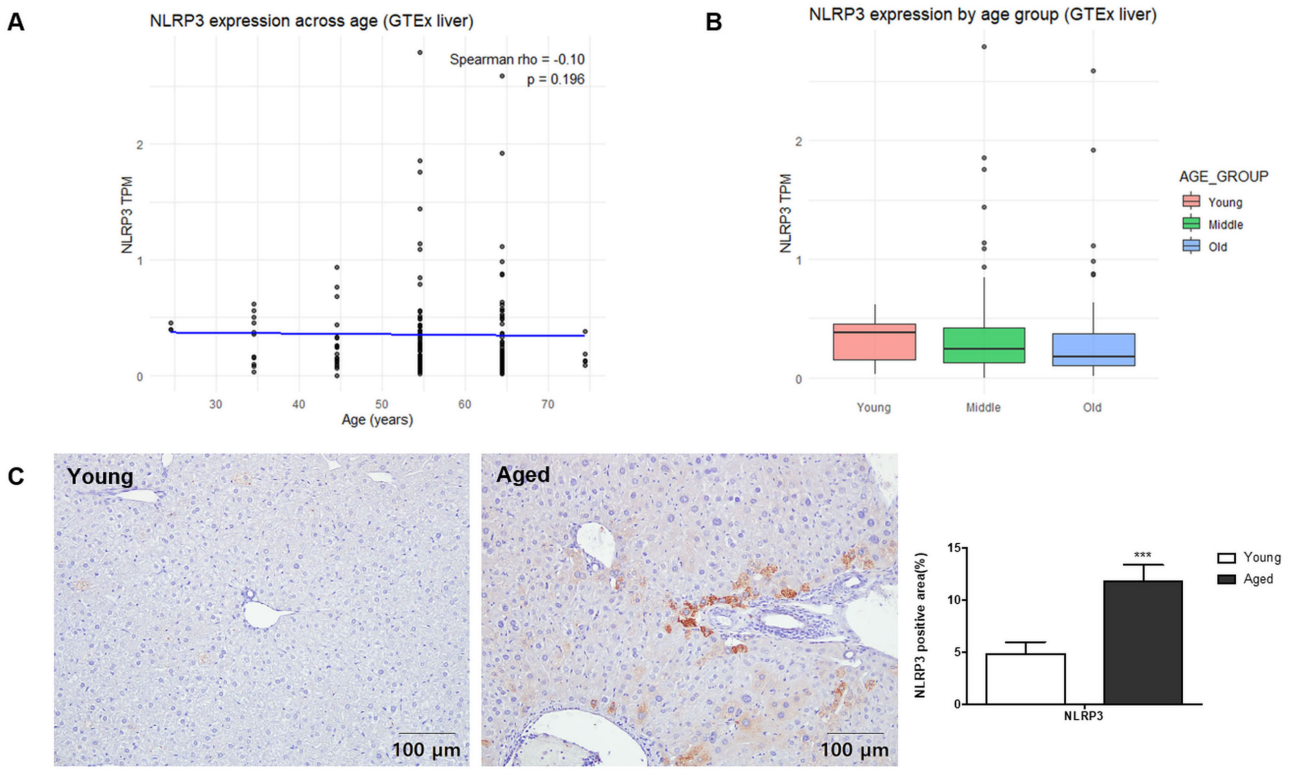

Fig. 1. Age-dependent hepatic NLRP3 expression in human and murine samples. (A) Correlation analysis of NLRP3 mRNA expression with chronological age in human liver samples using GTEx RNA-seq data. (B) Group-wise comparison of NLRP3 transcript levels among human donors classified into Young (<40 years), Middle (40–59 years), and Old (≥60 years) age groups. (C) Representative immunohistochemistry (IHC) images of NLRP3 in liver tissues from young and aged mice (left). Scale bar = 100 μm. Quantitative analysis of NLRP3-positive area based on IHC staining (right). Data are presented as mean ± SD. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. ***$p < 0.001$ versus the Young group. GTEx, Genotype-Tissue Expression; IHC, immunohistochemistry.

ing, membranes were incubated with the corresponding horseradish peroxidase–conjugated secondary antibodies. Immunoreactive signals were developed using a chemiluminescence-based detection reagent and captured with an image acquisition system (ATTO, Tokyo, Japan). Band intensities were quantified by densitometric analysis, and protein expression levels were normalized to the respective loading controls.

2.6 Statistical Analysis

All experiments were conducted in a double-blind manner. Results were randomly selected and expressed as the mean ± standard deviation (SD). GraphPad Prism (GraphPad Software, La Jolla, CA, USA) was used for all data analyses. The Mann-Whitney U test was used to assess differences in non-parametric data between two groups. For pairwise comparisons, a one-way analysis of variance (ANOVA) was performed, followed by a Tukey post hoc test when relevant. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Age-Associated Hepatic NLRP3 Expression in Humans and Mice

To investigate whether hepatic NLRP3 expression changes with aging, we first analyzed RNA-seq data from human liver samples in the GTEx cohort. NLRP3 transcript levels showed no significant correlation with chronological age (Spearman's $\rho = –0.10$, $p = 0.196$) (Fig. 1A). Consistently, stratification of donors into three age groups (Young <40 years, Middle 40–59 years, Old ≥60 years) revealed no significant differences in NLRP3 expression (Kruskal–Wallis $p > 0.05$) (Fig. 1B). These findings suggest that age-dependent alterations in hepatic NLRP3 expression may not be readily detectable at the transcriptomic level in heterogeneous human cohorts. We therefore examined NLRP3 expression in a spontaneously aging murine model. Immunohistochemical staining demonstrated markedly elevated NLRP3 protein levels in liver tissues from aged mice compared with young mice, with stronger cytoplasmic positivity and increased numbers of NLRP3-positive cells. Quantitative analysis confirmed a

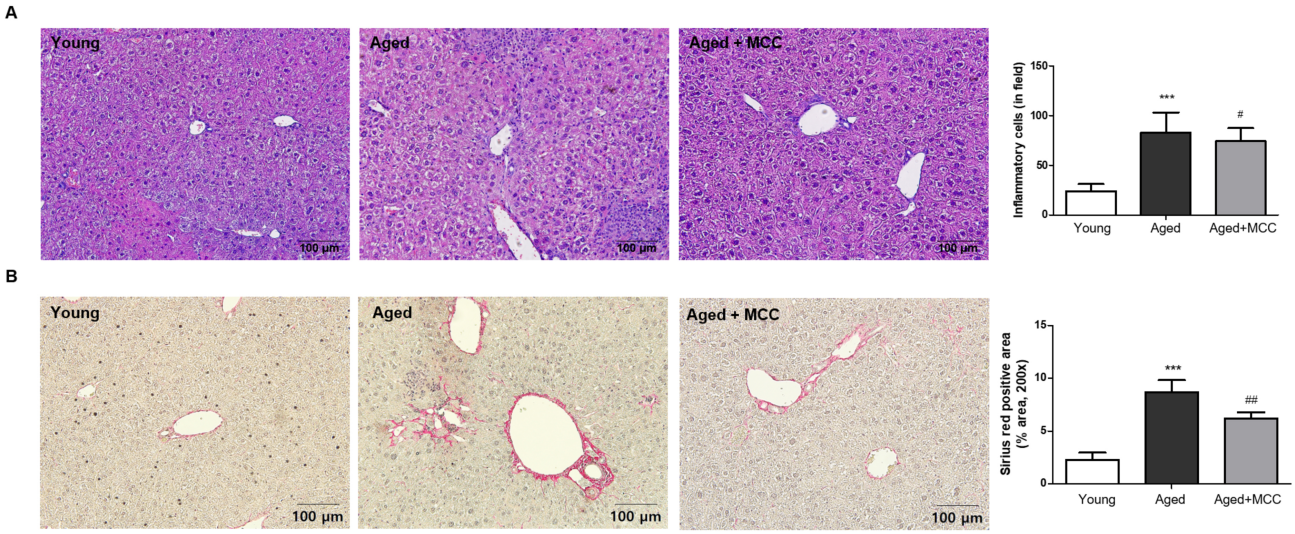

Fig. 2. MCC950 treatment attenuates age-associated hepatic inflammation and fibrosis. (A) Representative hematoxylin and eosin (H&E)-stained liver sections at 200× magnification (left) and quantitative analysis of inflammatory cell infiltration per field (right). (B) Representative Sirius Red–stained liver sections at 200× magnification (left) and quantitative assessment of collagen deposition (right). Experimental groups included Young (2 months, PBS, IP), Aged (18 months, PBS, IP), and Aged + MCC950 (18 months, 20 mg/kg MCC950, IP). Scale bars = 100 μm. Data are presented as mean ± SD. Statistical significance was analyzed using one-way ANOVA followed by Tukey's post hoc test. ***$p < 0.001$ versus the Young group; #$p < 0.1$ and ##$p < 0.01$ versus the Aged group. IP, intraperitoneal; PBS, phosphate-buffered saline.

significant increase in NLRP3-positive area in aged mouse livers (Fig. 1C). Together, these findings indicate that hepatic NLRP3 expression is enhanced during aging in mice, supporting a critical role for NLRP3 inflammasome activation in age-associated hepatic inflammation.

3.2 MCC950 Reduces Age-Related Hepatic Inflammation, Fibrosis, and Liver Injury

Given the increased hepatic NLRP3 protein expression in aged mice, we next examined the effects of MCC950 treatment on age-associated inflammatory and pathological changed in the liver. Aging was associated with pronounced inflammatory changes in the liver. H&E staining revealed substantial infiltration of inflammatory cells in aged mice, particularly around periportal and perisinusoidal regions, whereas young mice showed minimal inflammatory cell presence. Quantitative analysis confirmed a significant increase in inflammatory cell numbers in aged livers. Importantly, MCC950 treatment markedly reduced inflammatory cell infiltration, restoring liver architecture toward that of young controls (Fig. 2A). To assess age-associated fibrotic remodeling, Sirius Red staining was performed. Aged mice showed robust collagen deposition, with increased perivascular and periportal fibrosis. Sirius Red–positive area was significantly higher in aged livers, while MCC950 administration significantly reduced collagen accumulation, indicating attenuation of aging-related hepatic fibrosis (Fig. 2B). We next evaluated whether these histological improvements were accompanied by functional restoration. Serum ALT and AST levels were markedly elevated in aged mice, reflecting hepatocellular injury. MCC950 treatment significantly decreased both ALT and AST, although levels remained slightly above those of young mice (Fig. 3). These findings demonstrate that MCC950 mitigates age-related hepatic injury, improving both structural and biochemical indicators of liver damage.

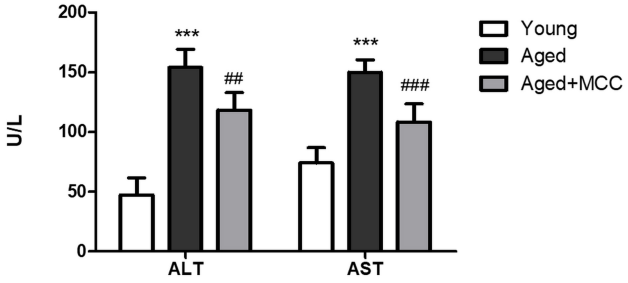

Fig. 3. Effects of MCC950 on serum liver injury markers in aged mice. Serum alanine aminotransferase (ALT) and Serum aspartate aminotransferase (AST) levels. Experimental groups were Young (2 months, PBS, IP), Aged (18 months, PBS, IP), and Aged + MCC950 (18 months, 20 mg/kg MCC950, IP). Data are presented as mean ± SD. Statistical significance was analyzed using one-way ANOVA followed by Tukey's post hoc test. ***$p < 0.001$ versus the Young group; ##$p < 0.01$ and ###$p < 0.001$ versus the Aged group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; IP, intraperitoneal.

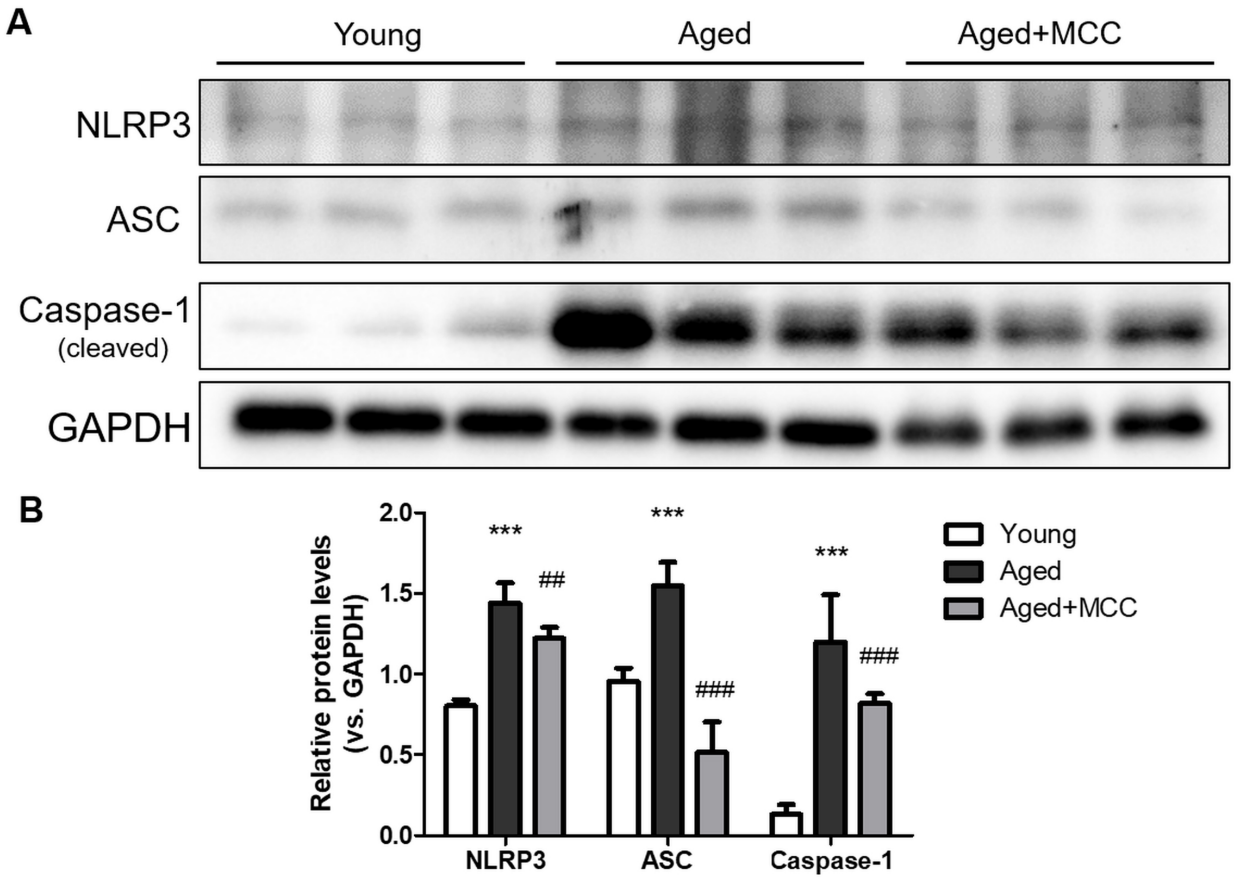

Fig. 4. NLRP3 inflammasome activation in aged liver and its suppression by MCC950. (A) Representative Western blot images showing hepatic protein expression of NLRP3, ASC, and cleaved caspase-1, with GAPDH as a loading control. (B) Densitometric quantification of the corresponding protein levels normalized to GAPDH. Experimental groups were Young (2 months, PBS, IP), Aged (18 months, PBS, IP), and Aged + MCC950 (18 months, 20 mg/kg MCC950, IP). Data are presented as mean ± SD. Statistical significance was analyzed using one-way ANOVA followed by Tukey's post hoc test. ***$p < 0.001$ versus the Young group; ##$p < 0.01$ and ###$p < 0.001$ versus the Aged group. ASC, apoptosis-associated speck-like protein containing a CARD; IP, intraperitoneal.

3.3 MCC950 Suppresses NLRP3 Inflammasome Activation in Aged Liver

We next investigated whether MCC950 effectively suppresses NLRP3 inflammasome signaling, the primary pharmacological target of this compound. Aged livers showed strong upregulation of NLRP3, ASC, and cleaved caspase-1, indicating increased inflammasome activation during aging. Quantification revealed significant increases in all three proteins in aged mice, whereas MCC950 markedly reduced NLRP3 and ASC levels and strongly suppressed caspase-1 activation (Fig. 4). These results showed that MCC950 effectively inhibits NLRP3 inflammasome activity in aged liver tissue.

3.4 MCC950 Decreases Downstream Inflammatory and Fibrogenic Signaling in Aged Liver

To further elucidate the mechanisms underlying the protective effects of MCC950 in aged liver, we examined downstream inflammatory and fibrogenic signaling pathways. Aged mice exhibited pronounced activation of inflammatory mediators, characterized by elevated IL-1β levels and robust phosphorylation of STAT3 (p-STAT3), indicating heightened cytokine-driven signaling during aging. Treatment with MCC950 significantly reduced both IL-1β expression and p-STAT3 levels, demonstrating effective suppression of downstream NLRP3-mediated inflammatory cascades (Fig. 5A,B). Consistent with these changes, immunohistochemical staining for the macrophage marker F4/80 revealed marked macrophage infiltration in aged livers, which was significantly attenuated by MCC950 administration (Fig. 5C,D). Given the observed reduction in collagen deposition and fibrosis, we next evaluated key markers of fibrogenic activation. Aged livers showed substantial increases in TGF-β1 and α-SMA expression, reflecting activation of hepatic stellate cells and progression of fibrotic pathways. Notably, MCC950 treatment markedly decreased TGF-β1 and α-SMA levels, indicating that inhibition of inflammasome signaling attenuates age-associated fibrogenic responses (Fig. 5E,F). Collectively, these findings indicate that MCC950 not only suppresses inflam-

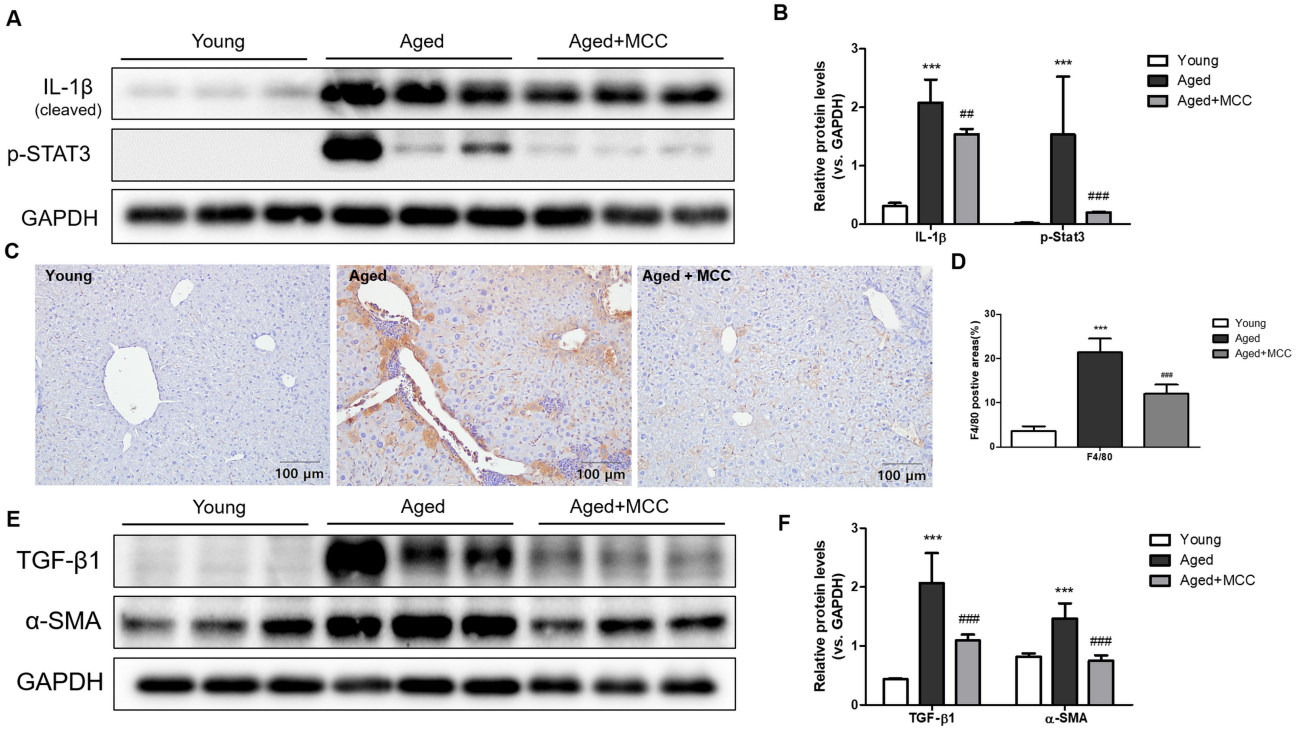

Fig. 5. MCC950 suppresses inflammatory and fibrogenic signaling in aged liver. (A) Representative Western blot images showing hepatic protein expression of the inflammatory markers IL-1β and phosphorylated STAT3 (p-STAT3), with GAPDH used as a loading control. (B) Densitometric quantification of IL-1β and p-STAT3 protein levels normalized to GAPDH. (C) Representative immunohistochemistry (IHC) images of F4/80 in liver tissues from each group. Scale bars: 100 µm. (D) Quantitative analysis of F4/80-positive area based on IHC staining. (E) Representative Western blot images showing hepatic protein expression of the fibrotic markers transforming growth factor beta 1 (TGF-β1) and α-smooth muscle actin (α-SMA), with GAPDH used as a loading control. (F) Densitometric quantification of TGF-β1 and α-SMA protein levels normalized to GAPDH. Experimental groups were Young (2 months, PBS, IP), Aged (18 months, PBS, IP), and Aged + MCC950 (18 months, 20 mg/kg MCC950, IP). Data are presented as mean $\pm$ SD. Statistical significance was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. ***$p < 0.001$ versus the Young group; ##$p < 0.01$ and ###$p < 0.001$ versus the Aged group. p-STAT3, phosphorylated signal transducer and activator of transcription 3; TGF-β1, transforming growth factor beta 1; α-SMA, alpha-smooth muscle actin.

matory signaling downstream of NLRP3 but also reduces macrophage infiltration and fibrogenic activation, thereby mitigating both inflammatory and structural deterioration in the aged liver.

3.5 MCC950 Restores Impaired Autophagy in Aged Liver

Given the known interplay between inflammasome activation and autophagy, we next evaluated whether MCC950 influences autophagy status in aged liver. Aged mice displayed profound impairment of autophagy, characterized by decreased expression of Vps34 and Atg14L, key components of the autophagosome initiation machinery. Additionally, aged liver exhibited a reduced LC3-II/LC3-I ratio and marked accumulation of p62, indicating suppressed autophagic flux. MCC950 treatment effectively reversed these autophagy defects, restoring Vps34 and Atg14L expression, increasing LC3-II/LC3-I ratio, and reducing p62 accumulation (Fig. 6). These findings suggest that suppression of NLRP3 inflammasome activity not only dampens inflammation and fibrosis but also reactivates autophagy pathways compromised during aging, contributing to improved hepatic homeostasis.

4. Discussion

In this study we demonstrate that aging is associated with increased NLRP3 inflammasome activation in the liver, which contributes to chronic low-grade inflammation and fibrotic susceptibility. We observed that aged mice exhibit higher NLRP3 expression, IL-1β production, and fibrogenic markers compared to young mice. Importantly, interventions that either inhibit NLRP3 or restore autophagy were able to mitigate these age-related changes in our models. These findings identify the NLRP3 inflammasome as a key driver of liver "inflammaging" and fibrosis, and they suggest that targeting inflammasome activity or bolstering autophagic clearance could help preserve liver health in the elderly.

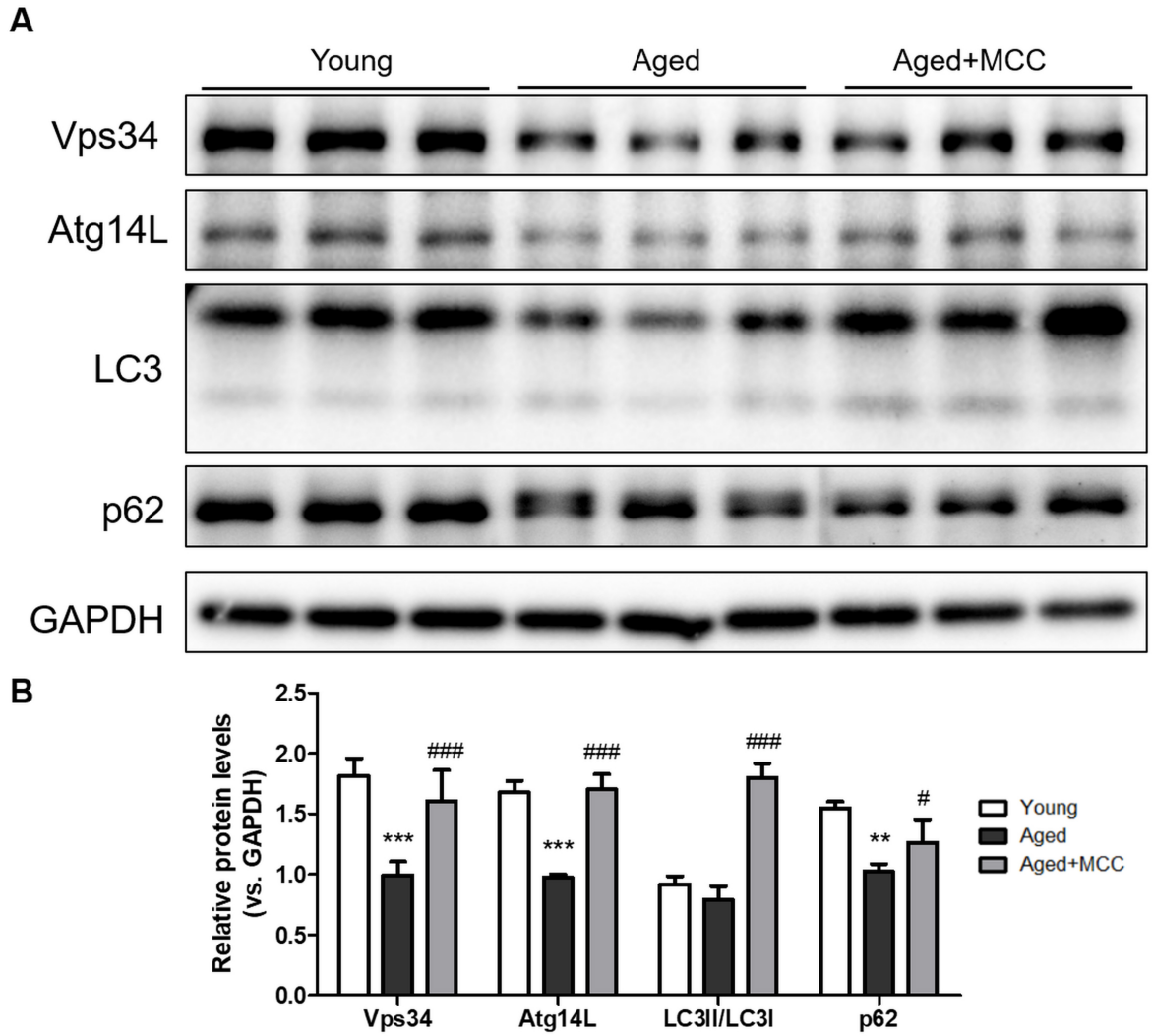

Fig. 6. MCC950 partially restores autophagy-related proteins in aged liver. (A) Representative Western blot images showing hepatic expression of autophagy-related proteins Vps34, Atg14L, LC3B, and p62, with GAPDH used as a loading control. (B) Densitometric quantification of the corresponding protein levels normalized to GAPDH. Experimental groups were Young (2 months, PBS, IP), Aged (18 months, PBS, IP), and Aged + MCC950 (18 months, 20 mg/kg MCC950, IP). Data are presented as mean ± SD. Statistical significance was analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{**}p < 0.01$ and $^{***}p < 0.001$ versus the Young group; $\#p < 0.01$ and $\#\#\#p < 0.001$ versus the Aged group. Vps34, vacuolar protein sorting 34; Atg14L, autophagy-related protein 14-like; LC3B, microtubule-associated protein 1 light chain 3 beta.

Aging fosters a pro-inflammatory milieu in the liver partly through the NLRP3 inflammasome [5]. Consistent with this, our findings show that aged livers exhibit elevated NLRP3 activation and inflammatory cytokine expression relative to young adults, which likely contributes to their increased susceptibility to liver injury and fibrosis. Prior studies similarly report that older mice develop more severe and persistent fibrosis than young mice following hepatic insults, coinciding with heightened NLRP3 levels, greater immune cell infiltration, and amplified fibrogenic activation [8]. Chronic metabolic conditions common in the elderly (such as NAFLD) may further exacerbate this pathway, as they lead to excessive NLRP3 activation and inflammatory tissue damage [5]. Taken together, the NLRP3 inflammasome emerges as a central mediator of age-related liver pathophysiology by driving ongoing inflammation and priming the liver for fibrotic remodeling.

Interestingly, our findings also highlight a divergence between human and murine aging with respect to NLRP3 expression. Specifically, analysis of the GTEx transcriptome data revealed no significant age-related increase in hepatic NLRP3 expression in healthy humans, consistent with previous reports indicating that sterile inflammation in human aging is often subtle and context-dependent [1,9]. However, it should be noted that GTEx-derived human transcriptomic data represent a heterogeneous population and may be influenced by multiple confounding factors, including underlying comorbidities, medication use, lifestyle

differences, and postmortem interval. Accordingly, the absence of a significant age-dependent change in hepatic NLRP3 mRNA levels in GTEx data should be interpreted with caution. This discrepancy may be attributable, at least in part, to regulatory processes beyond steady-state mRNA abundance (e.g., translational and protein turnover mechanisms) and to inherent differences between human donor-based transcriptomic datasets and controlled murine aging models. In the present study, GTEx analysis was used to provide contextual insight into human liver aging at the transcriptomic level, rather than to establish definitive causal relationships between aging and inflammasome activation. In contrast, our murine model exhibited a robust age-associated increase in hepatic NLRP3 protein expression, indicating more pronounced inflammasome activation during mouse liver aging, even in the absence of overt metabolic stress. These species-specific differences may reflect variations in immune surveillance, lifespan, or subclinical inflammatory burden, and underscore the inherent limitations of large-scale human postmortem datasets. Together, these findings highlight the value of complementary experimental models, such as spontaneously aged mice, for mechanistic investigation of age-related hepatic inflammation.

A major pathological consequence of NLRP3 inflammasome hyperactivation in the aging liver is the promotion of fibrosis. Persistent inflammasome signaling drives chronic release of IL-1β and IL-18, which not only sustains hepatic inflammation but also activates hepatic stellate cells (HSCs), thereby accelerating extracellular matrix deposition. In aged organisms, inflammatory resolution is often impaired, predisposing the liver to exaggerated and prolonged fibrotic remodeling after injury. Consistently, older mice and young mice with loss of the anti-aging factor SIRT1, which enhances NLRP3 activity—exhibit heightened inflammatory infiltration, sustained HSC activation, and increased collagen accumulation compared with young controls [8]. Importantly, disrupting this pathway can mitigate fibrogenesis: both genetic deletion and pharmacological inhibition of NLRP3 reduce inflammatory cytokine signaling and improve fibrotic outcomes, supporting NLRP3-driven inflammaging as a mechanistic link between age-associated inflammation and hepatic fibrosis [10–13].

Importantly, although pharmacological inhibition of NLRP3 with MCC950 has been reported to ameliorate liver inflammation and fibrosis in previous studies, most of these investigations were conducted in acute injury– or metabolically stressed models, such as toxin-induced or diet-induced liver disease [14]. In contrast, the present study specifically employed spontaneously aged mice, which develop hepatic inflammation and fibrotic susceptibility in the absence of overt exogenous insults. This distinction is critical, as spontaneous aging models more closely reflect the physiological and progressive nature of hepatic inflammaging, rather than secondary inflammatory responses driven by experimental injury. Therefore, our findings extend prior work by demonstrating that NLRP3 inflammasome activation is not merely a consequence of liver damage, but a primary driver of age-associated hepatic inflammation during natural aging.

Given its central involvement in age-related liver pathology, NLRP3 represents an attractive therapeutic target. MCC950, a highly selective small-molecule inhibitor of NLRP3, suppresses inflammasome assembly, thereby preventing caspase-1 activation and subsequent IL-1β maturation and release [15,16]. In aged mice, NLRP3 inhibition with MCC950 leads to reduced hepatic inflammation and improved tissue architecture. For example, Youm *et al*. [12] demonstrated that treating old, fibrogenic mice with MCC950 significantly attenuated liver inflammation and fibrosis, underscoring the inflammasome's role in driving these aging phenotypes. Our findings concur, as MCC950-treated old mice exhibited lower inflammatory cytokine levels and reduced fibrotic scar formation relative to untreated aged controls. These outcomes highlight the translational potential of NLRP3 inhibitors in managing age-associated liver disorders characterized by excessive inflammation.

Restoring autophagy represents a complementary therapeutic axis to inflammasome blockade because autophagic flux declines with aging, allowing damaged organelles and protein aggregates to accumulate and provide endogenous danger signals that can prime and activate the NLRP3 inflammasome. Conversely, autophagy acts as a negative regulator of NLRP3 signaling by removing inflammasome activators (e.g., damaged mitochondria) and, in some contexts, limiting inflammasome components and cytokine output [17]. Notably, rather than reflecting a secondary consequence of reduced inflammatory burden, our data indicate that MCC950 treatment preferentially restores components of the early autophagy initiation machinery. Consistent with this bidirectional crosstalk, our data show that MCC950-mediated suppression of IL-1β/inflammasome activation is accompanied by recovery of autophagy-related proteins in aged liver, including increased LC3 processing and normalization of p62, together with restoration of the autophagy nucleation machinery (VPS34–ATG14L complex). This linkage is biologically plausible, as prior work reported that MCC950 treatment in aged mice improves hepatic/metabolic dysfunction while inhibiting PI3K/Akt/mTOR signaling and enhancing autophagy, as reflected by increased LC3-II and reduced p62 [14] Moreover, because VPS34 and ATG14L are core constituents of the class III PI3K complex required for autophagosome nucleation, their recovery strongly supports reactivation of early autophagy steps, rather than a downstream change in cargo degradation alone [18]. Taken together, our findings suggest that MCC950 may interrupt a self-reinforcing inflammasome–autophagy loop in the aging liver: by dampening NLRP3/IL-1β output, MCC950

reduces inflammatory stress that otherwise impairs autophagic flux, thereby facilitating restoration of cellular quality-control pathways and promoting hepatic homeostasis during aging.

While our study provides important insights, several limitations should be acknowledged. First, murine findings may not directly translate to humans, as GTEx-based analyses suggest that age-related inflammasome changes in human liver are relatively subtle, warranting validation in elderly human cohorts. Second, the present study was conducted exclusively in male mice, which may limit the generalizability of our findings. Given well-documented sex-dependent differences in hepatic metabolism and inflammaging, future studies will incorporate female mice and apply appropriate experimental controls, such as estrous cycle staging or hormonal profiling, to comprehensively evaluate sex-specific effects. Third, MCC950 was administered for a relatively short duration in aged mice; therefore, the long-term efficacy, optimal treatment window, and safety of sustained NLRP3 inhibition remain to be determined. Finally, our analyses were largely performed at the whole-tissue level, and future work using cell-type–resolved approaches (e.g., single-cell profiling or conditional knockouts) will be required to define the principal hepatic cell populations driving NLRP3 activation and IL-1β production during aging.

5. Conclusions

Taken together, our results indicate that targeting the NLRP3 inflammasome while restoring autophagy is an effective strategy to counter age-associated hepatic inflammation and fibrosis. By simultaneously reducing inflammasome-driven cytokine output and reactivating cellular quality-control pathways, this approach disrupts the self-perpetuating cycle of hepatic inflammaging. Although additional studies are required to establish translational applicability, our findings provide clear proof-of-concept that coordinated modulation of NLRP3 signaling and autophagy can improve liver homeostasis in aging.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding authors upon request.

Author Contributions

KHK: Writing-original draft, Animal modeling, Investigation, Review & Editing. YWS: Animal modeling, Investigation. YJK: Animal modeling, Investigation. HJL: Animal modeling, Investigation. JLR: Investigation. HTK: Animal modeling. GLH: Investigation. JYJ: Conceptualization, Funding acquisition, Supervision, Project Administration. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal experimental protocols were approved by the Institutional Animal Ethics Committee of Chungnam National University (approval number: 202307A-CNU-136). This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals of National Institutes of Health.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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