

Curcumin ameliorates cerulein-induced chronic pancreatitis through Nrf-2/HO-1 signaling

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Abstract. Chronic pancreatitis (CP) is an invasive inflammatory disorder characterized by endocrine and exocrine dysfunction. There are currently no effective drugs for the treatment of CP. The present study investigated whether curcumin improves cerulein-induced CP fibrosis in a mouse model and pancreatic stellate cells (PSCs). The CP mouse model was established by intraperitoneally injecting cerulein (50 µg/kg) for 3 weeks (six times at 1 h intervals/day; 4 days/week). To investigate the effects of curcumin, dimethyl sulfoxide or curcumin was injected intraperitoneally 1 h before the first daily injection of cerulein. To determine the severity of CP, the pancreas was harvested 24 h after the last cerulein injection for histological examination and assessment of PSC activation and collagen deposition. Additionally, levels of the nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) were evaluated to determine the mechanism underlying the anti-fibrotic effect of curcumin in PSCs. Curcumin improved pancreatic injury associated with CP by inhibiting PSC activation and collagen deposition. Moreover, curcumin increased HO-1 expression levels via the activation

of Nrf2 in PSCs, which suppressed the activation of PSCs. In conclusion, the present results suggest that curcumin can ameliorate pancreatic fibrosis induced by repetitive cerulein challenges via the induction of HO-1 and is a beneficial agent for the treatment of CP.

Introduction

Chronic pancreatitis (CP) is a painful disease of the exocrine pancreas leading to exocrine insufficiency and characterized by inflammatory cell infiltration, parenchymal atrophy and extensive fibrosis of the exocrine pancreas (1,2). CP is typically caused by excessive drinking, smoking, drugs, genetics and toxic metabolites; however, the exact cause has not been identified (3). Treatment for CP is currently limited to improving the quality of life through pain relief and digestive enzyme supplementation; therefore, there is need to develop an effective treatment strategy (4,5). Although the pathophysiology of CP is not well understood, several hypotheses have been studied (6-8). Repetitive acute inflammation of the pancreas without recovery time for a damaged pancreas leads to the activation of the fibrotic cascade (9). Following an initial episode of acute pancreatitis (AP), persistent inflammation elicits immune cell infiltration and activates pancreatic stellate cells (PSCs) (10,11). Although quiescent under normal conditions, PSCs are activated after pancreatic injury and transform into a myofibroblast-like α -smooth muscle actin (SMA)-positive cell type that actively proliferates, produces extracellular matrix (ECM) and secretes growth factors (12-15). Activated PSCs serve a key role in pancreatic fibrosis and are emerging as an important target for the treatment of CP (6,16,17).

Curcumin, the primary polyphenolic compound derived from the rhizomes of *Curcuma longa*, has been reported to exhibit anti-inflammatory, antioxidant, antimicrobial and anti-tumor activity in various disease models (18-21). Additionally, curcumin and *C. longa* have protective effects against AP, which may cause CP (22,23). Curcumin inhibits PSC proliferation by inducing heme oxygenase (HO)-1 (24,25). However, the aforementioned studies on pancreatic fibrosis using curcumin

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used an *in vitro* model using isolated rat PSCs. Therefore, we need to investigate the effect of curcumin on CP using *in vivo* and *in vitro* models in mice.

The present study evaluated the antifibrotic effects of curcumin against cerulein-induced CP in mice. To evaluate the severity of CP, histological changes, PSC activation and collagen deposition were assessed in the pancreas of a murine CP model. Additionally, PSCs were isolated to assess the regulatory mechanisms of curcumin.

Materials and methods

Materials. Cerulein, curcumin and trigonelline were purchased from MilliporeSigma. The monoclonal antibodies against nuclear factor erythroid 2-related factor 2 (Nrf2; cat. no. sc-365949) and α -smooth muscle actin (SMA; cat. no. sc-32251) were purchased from Santa Cruz Biotechnology, Inc. The antibody against collagen I (ab34710) was purchased from Abcam. The antibody against heme oxygenase (HO)-1 (70081S) was purchased from Cell Signaling Technology, Inc. The TRIzol reagent and High-Capacity RNA-to-cDNA™ Kit were purchased from Thermo Fisher Scientific, Inc.

Animal model. All experiments were performed according to the protocols of the Animal Care Committee of Wonkwang University and approved by the Institutional Animal Care and Use Committee Certification of Wonkwang University, South Korea (approval no. WKU 25-4). C57BL/6 female mice (age, 6–8 weeks; weight, 15–20 g; n=63 mice) were purchased from ORIENT BIO, INC. All animals were bred and housed in standard shoebox cages in a climate-controlled room with an ambient temperature of $23\pm 2^{\circ}\text{C}$, humidity of $50\pm 5\%$, and a 12/12-h light-dark cycle. The animals were fed standard laboratory chow, allowed water *ad libitum* and randomly assigned to either the control or experimental group (n=9/group). CP was induced by intraperitoneal injection of a supramaximal concentration of the stable cholecystokinin analog cerulein ($50\text{ }\mu\text{g/kg}$), administered six times/day at 1-h intervals, and repeated four times/week for a total of 3 weeks. The animals in the control group were administered saline instead of cerulein under the same conditions. Curcumin (10 or 20 mg/kg) or DMSO (control) was administered intraperitoneally 1 h before the first cerulein injection to the experimental and control groups, respectively. Mice were sacrificed 24 h after the last cerulein injection. Isoflurane (induction, 4.5; maintenance; 1.5%) in 95% O_2 and 5% CO_2 was used for anesthesia. CO_2 inhalation was used for euthanasia with a flow rate that displaced 50% of the cage vol/min and cervical dislocation was also performed to ensure death following CO_2 asphyxiation. The pancreatic samples were immediately collected for further examination.

Histological analysis. For histological examination and scoring, the pancreas tissue was fixed in 4% formalin solution for overnight at room temperature, embedded in paraffin, cut into $4\text{-}\mu\text{m}$ sections, stained with hematoxylin-eosin (H&E) and examined under a light microscope. H&E staining was performed as follows: Hematoxylin for 8 min and eosin for 2 min at room temperature. The pancreases were analyzed

in a blinded manner and graded using a semi-quantitative scoring system for edema, loss of acini and inflammatory cell infiltration. The samples were scored on a scale from 0 to 3 based on the presence of glandular atrophy and inflammation (0, normal, no glandular atrophy and inflammation; 1, mild, found in less than 25% of the pancreas. 2=moderate, found in less than 25 to 75% of the pancreas. 3=found in more than 75% of the pancreas). For Sirius Red staining, paraffin-embedded pancreatic sections ($4\text{-}\mu\text{m}$ thick) were stained with 0.1% Sirius Red F3B in saturated picric acid for 1 h at room temperature (Sigma-Aldrich; Merck KGaA).

Immunofluorescence analysis. For immunofluorescence staining, tissues were sectioned at $9\text{ }\mu\text{m}$ thickness. The slides were blocked with blocked with serum (1% BSA; BSAS0.1; Bovogen Biologicals) at RT for 1 h and stained overnight with primary antibodies against α -SMA (1:1,000) and collagen I (1:1,000) at 4°C , followed by treatment with Alexa Fluor®594-labeled secondary antibody (1:2,000; A11012, A11005; Invitrogen; Thermo Fisher Scientific, Inc.) at room temperature for 1 h. Nuclei were counterstained with DAPI for 5 min at room temperature. Stained sections were visualized under a confocal laser microscope (Olympus Corporation).

Reverse transcription-quantitative PCR (RT-qPCR). mRNA transcripts in pancreatic tissue were analyzed using RT-qPCR. Total RNA was isolated using TRIzol reagent according to the manufacturer's instructions. Total RNA ($1\text{ }\mu\text{g}$) was converted to cDNA using a High-Capacity RNA-to-cDNA™ kit (4387406; Applied Biosystems; Thermo Fisher Scientific, Inc.) at 37°C for 60 min and 95°C for 5 min. RT-qPCR was carried out using TaqMan™ Universal Master Mix II, no UNG (Applied Biosystems; Thermo Fisher Scientific, Inc.) on ABI StepOnePlus detection system according to the manufacturer's instructions (Applied Biosystems; Thermo Fisher Scientific, Inc.). The conditions for qPCR were as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of amplification at 95°C for 10 sec and 60°C for 30 sec. The expression of the genes of interest were analyzed in triplicate and included a control reaction in which reverse transcriptase was not added to the reaction mixture. Relative gene expression (target gene expression normalized to that of the endogenous control gene) was calculated using the comparative Cq method (26). The results were normalized to those of the housekeeping gene hypoxanthine-guanine phosphoribosyltransferase (Hprt). Forward, reverse and probe oligonucleotide primers were purchased from Applied Biosystems (Thermo Fisher Scientific, Inc.; actin $\alpha 2$ (Acta2), cat. no. Mm01546133_m1; fibronectin 1 (Fn1), cat. no. Mm01256734_m1; collagen, type I, alpha 1 (Col1a1), cat. no. Mm00801666_g1; collagen, type IV, alpha 1 (Col4a1), cat. no. Mm01210125_m1, transforming growth factor beta (Tgfb), cat. no. Mm00441726_m1 and Hprt, cat. no. Mm03024075_m1). The sequences of the primers are not commercially available.

PSC isolation. Mouse PSCs were prepared from pancreatic tissue as previously described (7). The pancreas was immediately cut into small pieces with scissors and digested for 20 min at 37°C in a shaking water bath using Gey's balanced salt solution (GBSS) containing collagenase, filtered through

a 100- μ m nylon mesh and subjected to isopycnal separation with Nycodenz solution (D2158-100G; Sigma Aldrich). The PSCs were collected from the upper layer of the gradient, washed with GBSS and cultured in DMEM, high glucose, pyruvate (11995; Gibco™) containing 10% fetal bovine serum (cat. no. 16000-044; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin. The isolated PSCs were incubated at 37°C with 5% CO₂, and the cells from passages 3 to 5 were used for further experiments. PSCs were cultured in serum-free DMEM for 24 h before treatment with the experimental reagents at 37°C. To investigate the effect of curcumin on HO-1, PSCs were treated with curcumin (1, 5, 10 or 20 μ M) for 6, 9, 12 or 24 h. PSCs were treated with 10 μ M cobalt protoporphyrin (CoPP) for 6 h as a positive control for HO-1 expression. To investigate the effect of curcumin on the Nrf2/HO-1 pathway, PSCs were treated with 10 μ M curcumin for 6 h in the presence or absence of trigonelline 10 μ M pretreated and then treated with curcumin. To investigate whether curcumin-induced HO-1 was associated with the antifibrotic effect in PSCs, PSCs were treated with or without pretreatment with 10 μ M tin protoporphyrin-IX (SnPP) and curcumin to determine the expression of HO-1. Next, PSCs were divided into groups treated with 10 μ M curcumin for 1 h and 0.5 ng/ml TGF- β 1 for 24 h.

Western blotting. Mouse PSCs were lysed on ice with radioimmunoprecipitation assay lysis buffer (iNtRON Biotechnology). Then, the lysates were boiled in 62.5 mM Tris-HCl buffer, pH 6.8, containing 2% SDS, 20% glycerol and 10% 2-mercaptoethanol. Protein samples were quantified using BSA and were loaded into each well (20 μ g) and separated by 10% SDS-PAGE and transferred onto a nitrocellulose membrane. Membranes were blocked with 5% skimmed milk in PBS-0.1% Tween-20 (PBST) for 2 h at room temperature and incubated overnight with antibodies against HO-1 (1:1,000) for overnight at 4°C. After washing three times with PBST, each blot was incubated with peroxidase-conjugated secondary antibody (1:5,000; SA002-500; GenDEPOT) for 1 h at room temperature. The proteins were visualized using an enhanced chemiluminescence detection system (GE Healthcare) according to the manufacturer's protocols. The bands were detected and quantified by using Quantity One software (version 4.5.2; Bio-Rad Laboratories, Inc.).

Immunofluorescence analysis of mouse PSCs. Mouse PSCs were plated at density of 1×10^5 cells/well in a chamber slide and incubated with 10 μ M curcumin for 6 h at 37°C. The cells were fixed in 4% paraformaldehyde for 15 min at room temperature and washed thrice with PBST. The cells were treated with 0.1% Triton X-100 for 15 min at room temperature. After washing with PBST, non-specific binding sites were blocked with serum (1% BSA) for 1 h at room temperature and incubated overnight with Nrf2 antibody at 4°C (1:1,000). The cells were washed with PBST and incubated with AlexaFluor®594 secondary antibody (1:2,000; A11012; Invitrogen) for 2 h at room temperature in the dark. For nuclear staining, the cells were incubated with DAPI (5 mg/ml) for 5 min at room temperature. The slides were washed with PBST and mounted for examination under a confocal laser microscope (Olympus Corporation).

Statistical analysis. Data are expressed as mean \pm standard error of the mean. Statistical significance was evaluated using one-way analysis of variance. Post-hoc analysis using the Duncan method for multiple comparisons among groups. $P < 0.05$ was considered to indicate a statistically significant difference. All experiments were conducted in triplicate.

Results

Curcumin ameliorates pancreatic injury by cerulein-induced CP. To evaluate the effect of curcumin on CP, mice were intraperitoneally administered either DMSO (control) or curcumin (10 or 20 mg/kg) 1 h before the first administration of cerulein (Fig. 1A). Following CP induction, H&E staining was conducted to investigate histological changes in the pancreas. The pancreas of CP mice was characterized by the loss of acinar cells, inflammatory cell infiltration and edema, which were suppressed by curcumin treatment (Fig. 1B). The curcumin treatment group showed a dose-dependent decrease in the edema, inflammation, and glandular atrophy compared to the CP group (Fig. 1C).

Curcumin inhibits PSC activation and collagen deposition associated with CP. CP is typically accompanied by pancreatic fibrosis due to activation of PSCs, which express α -SMA and produce ECM components, such as collagen and fibronectin (6,7). Therefore, the activation of PSCs in the pancreas was evaluated using immunofluorescence staining of α -SMA. A marked increase of α -SMA-positive PSCs was displayed in tissue from cerulein-induced CP mice. However, curcumin treatment significantly decreased the α -SMA-positive area of pancreatic tissue (Fig. 2A and B). RT-qPCR was performed to examine the mRNA expression of α -SMA. The elevated mRNA expression levels of Acta2 (which encodes α -SMA) associated with CP was decreased by curcumin treatment (Fig. 2C).

Next, the effects of curcumin on ECM production in activated PSCs were investigated. Sirius Red staining was performed to evaluate the production and deposition of collagen, which is a representative ECM component (27). In the cerulein-induced CP group, collagen production and deposition increased significantly, whereas in the curcumin-treated group, collagen production and deposition decreased in a concentration-dependent manner (Fig. 3A and B). Collagen I levels were evaluated using immunofluorescence staining. The collagen I-positive area significantly increased in the cerulein-induced CP group but decreased in the curcumin-treated group (Fig. 3C and D). In addition, curcumin decreased the mRNA expression levels of ECM components, such as Colla1, Col4a1 and Fn1, which were increased in cerulein-induced CP samples (Fig. 3E-G).

Curcumin activates the Nrf2/HO-1 pathway in isolated PSCs. Curcumin affects inflammation, oxidative stress and fibrosis via the Nrf2/HO-1 pathway (19,28). Therefore, PSCs were isolated from the mouse pancreas to investigate the effects of curcumin. The changes in the mRNA and protein expression of HO-1 in PSCs following exposure to curcumin were examined. PSCs were treated with curcumin at different doses (1, 5, 10 or 20 μ M) for 6, 9, 12 or 24 h. PSCs were treated with 10 μ M

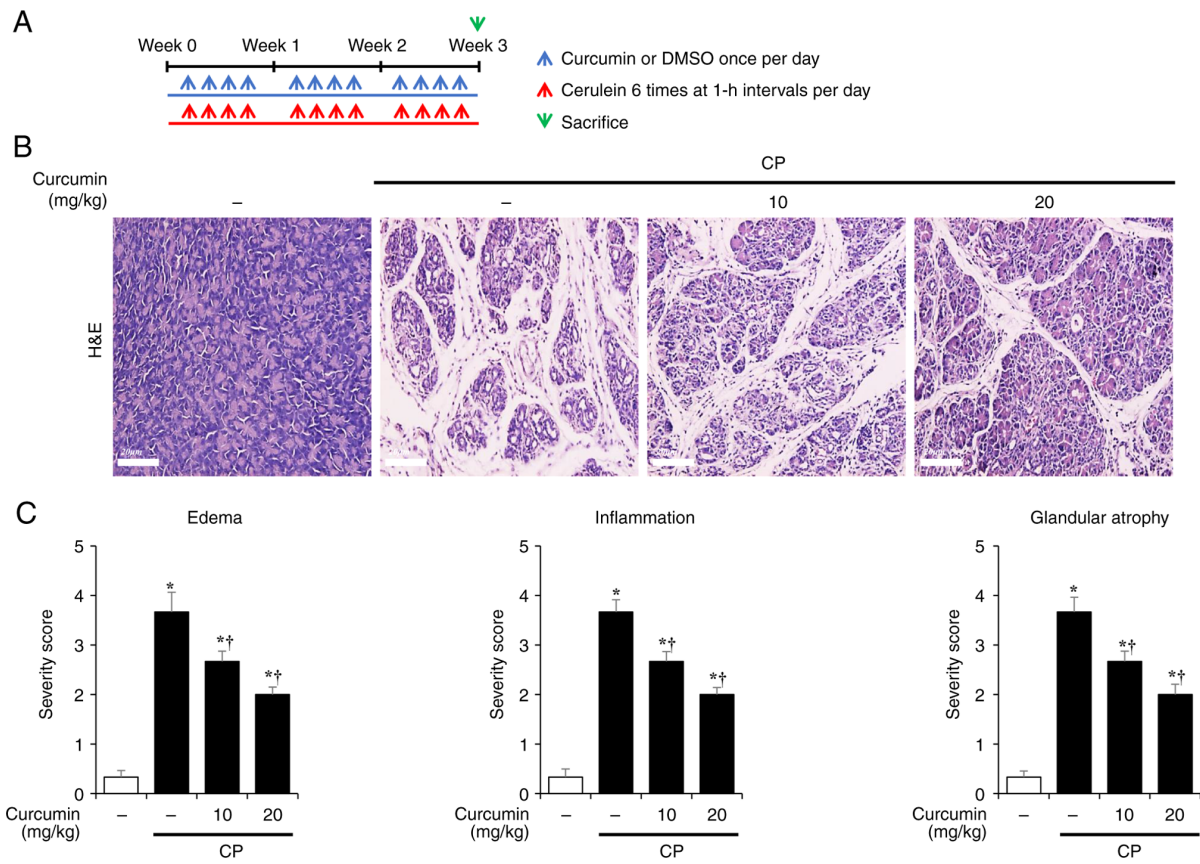


Figure 1. Effect of curcumin on the histopathology of the pancreas during CP. (A) CP mouse model to examine the prophylactic effect of curcumin. (B) Representative H&E-stained sections of the pancreas. (C) Histological sections of the pancreas were scored 0 (normal) to 3 (severe) for glandular atrophy, edema and inflammation. * $P < 0.05$ vs. DMSO; † $P < 0.05$ vs. CP. Magnification, $\times 200$. Scale bar, $20 \mu\text{m}$. CP, chronic pancreatitis; H&E, hematoxylin and eosin.

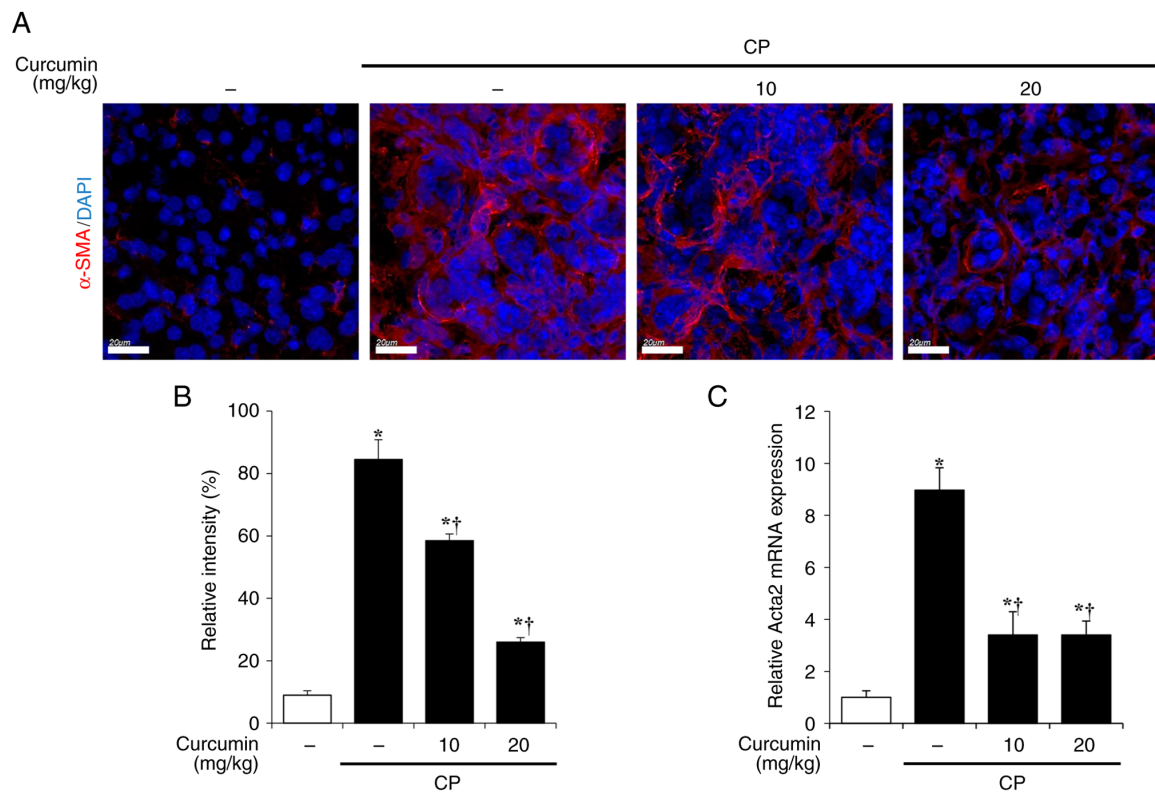


Figure 2. Effect of curcumin on PSC activation associated with CP. (A) Immunofluorescence staining of α -SMA (red) in the pancreas. (B) Relative intensity of α -SMA staining. (C) mRNA expression of Acta2 in the pancreas measured by reverse transcription-quantitative PCR. * $P < 0.05$ vs. DMSO; † $P < 0.05$ vs. CP. Magnification, $\times 400$. Scale bar, $20 \mu\text{m}$. CP, chronic pancreatitis; PSC, pancreatic stellate cell; α -SMA, α -smooth muscle actin; Acta2, actin $\alpha 2$.

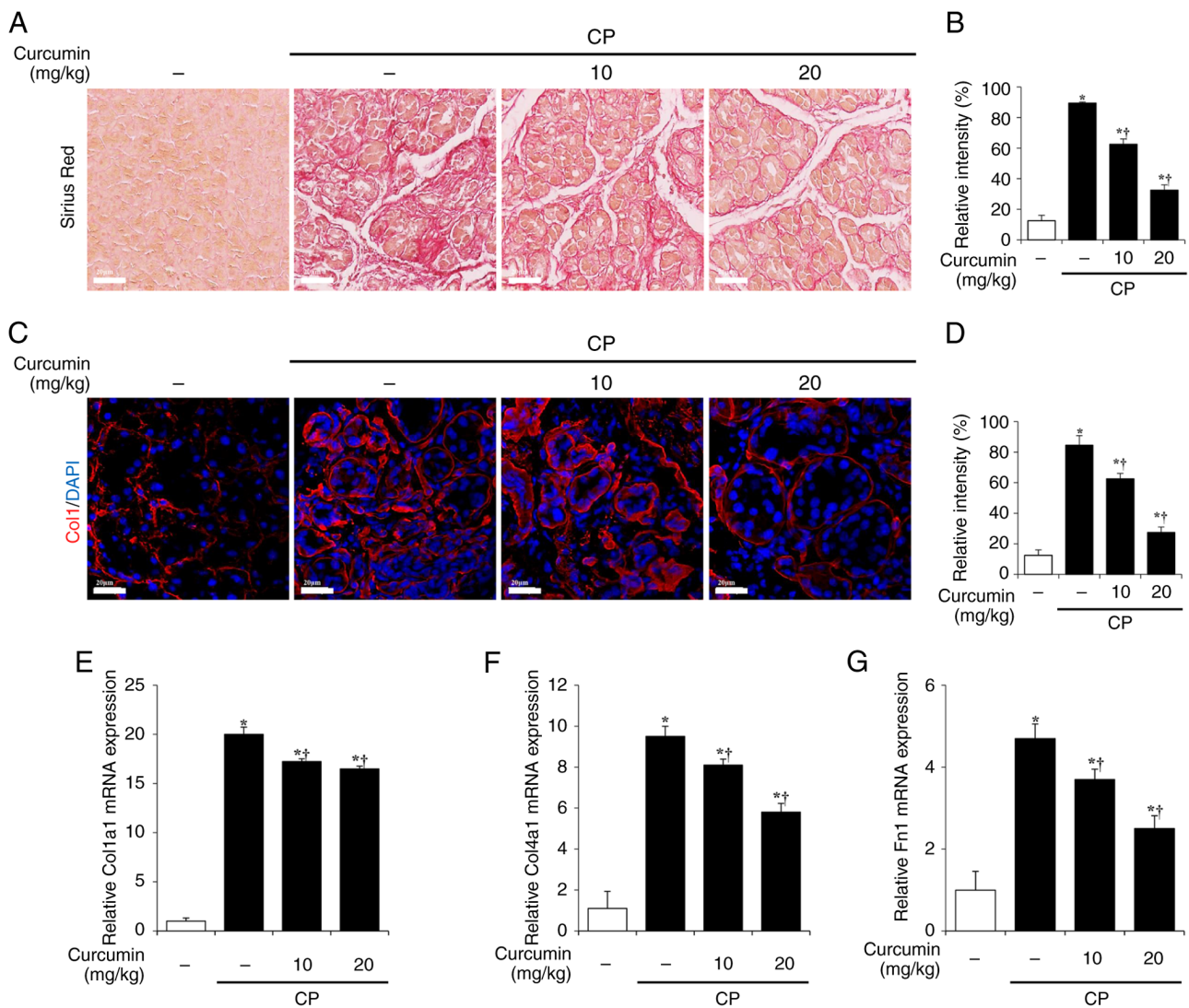


Figure 3. Effect of curcumin on extracellular matrix deposition associated with CP. (A) Sirius Red staining of collagen deposition (red) in the pancreas. (B) Relative intensity of Sirius Red staining. (C) Immunofluorescence staining of Col1 (red) in the pancreas. (D) Relative intensity of collagen I. mRNA expression levels of (E) Col1a1, (F) Col4a1 and (G) Fn1 in the pancreas measured by reverse transcription-quantitative PCR. * $P < 0.05$ vs. DMSO, † $P < 0.05$ vs. CP. Magnification, $\times 400$. Scale bar, $20 \mu\text{m}$. CP, chronic pancreatitis; Col1a1, collagen type IV, alpha 1; Fn1, fibronectin1.

CoPP for 6 h as a positive control for HO-1 expression. mRNA expression of HO-1 in PSCs increased following 6 h treatment with 5 and $10 \mu\text{M}$ curcumin and decreased at $20 \mu\text{M}$ curcumin (Fig. 4A). In addition, HO-1 mRNA expression significantly increase after 3 h treatment with $10 \mu\text{M}$ curcumin, reached its highest level after 6 h and decreased after 9 and 24 h. (Fig. 4B).

To investigate changes in the protein expression levels of HO-1, PSCs were treated with $10 \mu\text{M}$ curcumin for 1, 2, 3, 6, 9 or 24 h. HO-1 protein expression began to increase from 3 h after curcumin treatment, reached the highest level at 9 h, and showed a tendency to slightly decrease after 24 h. (Fig. 4C). As curcumin is known to produce HO-1 via the Nrf2/HO-1 pathway (19,28), changes in Nrf2 expression in PSCs were investigated. Nrf2 exists in the cytoplasm; however, when activated, it translocates to the nucleus, binds antioxidant-responsive elements (ARE) and transcribes related factors such as HO-1, NAD(P)H quinone oxidoreductase 1 and superoxide dismutase (29). To determine whether curcumin increased expression of HO-1 via the Nrf2/HO-1

pathway, PSCs were pretreated with $10 \mu\text{M}$ trigonelline, an Nrf2 inhibitor, for 6 h and curcumin ($10 \mu\text{M}$ for 6 h). In the DMSO-treated control group, Nrf2 was present in the cytosol; however, in the curcumin-treated group, it was activated and translocated to the nucleus (Fig. 4D). Additionally, pretreatment with trigonelline decreased the mRNA expression levels of HO-1, which were increased by curcumin (Fig. 4E).

Curcumin regulates PSC activation and ECM production by inducing HO-1 in isolated PSCs. Whether curcumin affects PSC activation and ECM production in PSCs by inducing HO-1 production was investigated. The effect of curcumin on HO-1 expression was investigated after treatment with SnPP, a HO-1 inhibitor. PSCs were pretreated in the presence or absence of SnPP ($10 \mu\text{M}$) for 1 h and treated with curcumin ($10 \mu\text{M}$). After 24 h, HO-1 expression was evaluated at the protein level. When the PSCs were treated with curcumin ($10 \mu\text{M}$) alone, HO-1 expression increased; however, in the group pretreated with SnPP, the increase in HO-1 caused

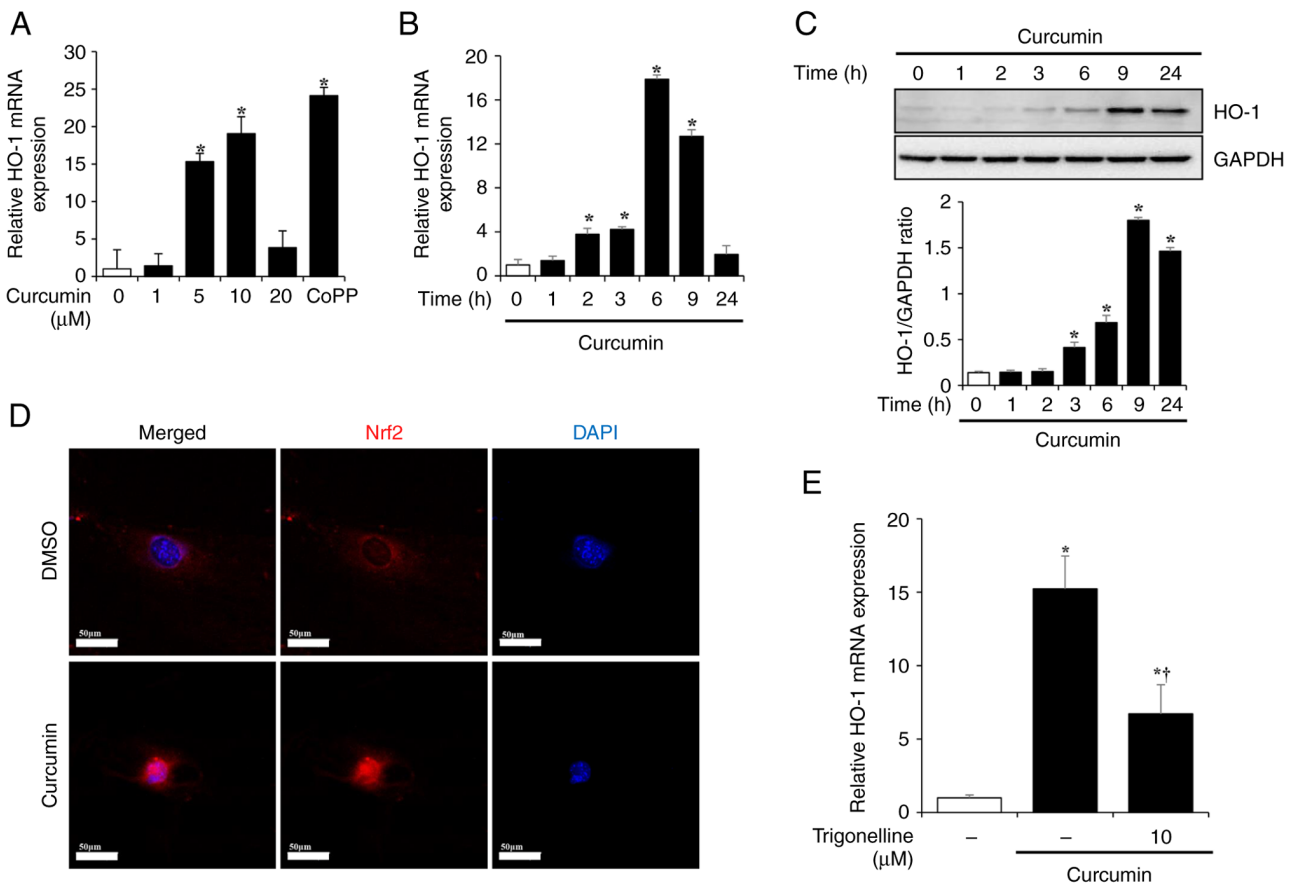


Figure 4. Effect of curcumin on HO-1 expression in PSCs. (A) Dose- and (B) time-dependent effect of HO-1 on mRNA expression. (C) Time-dependent effect of curcumin on HO-1 protein expression. (D) Immunofluorescence staining of Nrf2 (red) in isolated PSCs. (E) mRNA expression of HO-1 was assessed by reverse transcription-quantitative PCR. * P <0.05 vs. DMSO; † P <0.05 vs. curcumin. Magnification, x600. Scale bar, 50 μ m. HO-1, heme oxygenase-1; PSC, pancreatic stellate cell.

by curcumin was suppressed (Fig. 5A). This suggested that SnPP treatment effectively suppresses the effect of curcumin on HO-1 expression. Next, whether curcumin-induced HO-1 affected PSC fibrosis was investigated. PSCs were treated with TGF- β 1 (0.5 ng/ml for 24 h) for activation and the markers of PSC activation and ECM production (Acta2, Tgfb1, Colla1 and Col4a1) were measured. Increased expression following TGF- β 1 treatment and a significant decrease in expression of all factors following curcumin treatment were observed. However, in the SnPP-pre-treated group, the inhibitory effects of curcumin on PSC activation and ECM production were reversed (Figs. 5B-D).

Discussion

Curcumin is used as a spice and is known to have pharmacological effects such as wound healing, antidiabetic, antimicrobial and anti-inflammatory effects (30). Additionally, previous studies have revealed the therapeutic effects of curcumin against numerous diseases including cancer, cardiovascular disease, psoriasis, diabetes and peptic ulcers (31,32). However, to the best of our knowledge, the effect of curcumin on CP has not been studied. Therefore, the present study examined the protective effects of curcumin against cerulein-induced CP in mice.

CP is an inflammatory fibrotic disease accompanied by pancreatic dysfunction and severe abdominal pain that causes

irreversible damage to the pancreatic tissue (33,34). Patients can recover from a single AP episode, but repeated AP exposure can develop into CP (35). The 3-week cerulein-induced pancreatitis model used in the present study was based on repetitive AP that leads to an increase in inflammatory cells, tissue damage (edema and pancreatic cell depletion) and fibrosis in the pancreas via inflammatory mediators, cytokines and oxidative free radicals (36). In the present study, histological characteristics of CP, such as pancreatic atrophy, loss of acinar cells, edema and influx of inflammatory cells, were observed. However, curcumin administration suppressed pancreatic histological damage.

Pancreatic fibrosis is a characteristic of CP. PSCs carry out an important role in fibrosis progression (37). In the normal pancreas, PSCs account for 4% of the total pancreatic tissue; when activated, they proliferate and produce ECM (14,38). Therefore, the inhibition of PSC activation and ECM production may be an effective therapeutic strategy against CP. In the present study, PSC activation was evaluated by α -SMA staining and ECM production by collagen staining in the pancreas. In the CP group, the areas that stained positive for α -SMA and collagen were notably increased, whereas they decreased in the curcumin-treated group. In addition, curcumin reduced the mRNA levels of the fibrosis-associated factors Acta2, Colla1, Col4a1 and Fn1, which increased in the CP group.

HO-1 exerts antioxidant effects, as well as pharmacological effects, such as anti-inflammatory, anti-fibrotic,

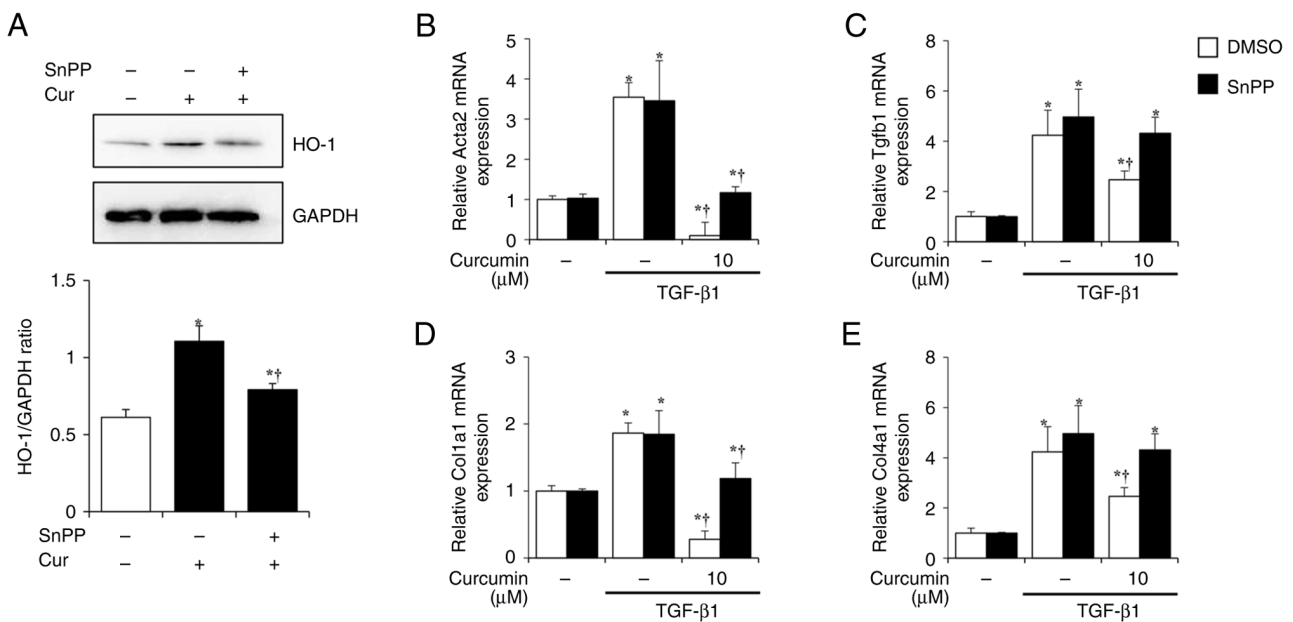


Figure 5. Effect of curcumin-induced HO-1 on PSC activation and extracellular matrix production. (A) Protein levels of HO-1 following curcumin treatment with or without SnPP in isolated PSCs measured by western blotting. * $P < 0.05$ vs. DMSO; † $P < 0.05$ vs. Curcumin. mRNA expression levels of (B) Acta2, (C) Tgfb1, (D) Col1a1 and (E) Col4a1 measured by reverse transcription-quantitative PCR. * $P < 0.05$ vs. DMSO; † $P < 0.05$ vs. TGF- β 1. HO-1, heme oxygenase-1; PSC, pancreatic stellate cell; SnPP, tin protoporphyrin IX; Acta2, actin alpha 2; Col1a1, collagen, type IV, alpha 1.

anti-obesity and anti-dementia activity (39-42). There are numerous mechanisms that increase HO-1 production, such as the Nrf2/HO-1, c-Jun/activated Protein 1 (AP1)/HO-1 and NF- κ B/HO-1 pathways (43,44). Nrf2 is a transcription factor responsible for regulating the cellular redox balance. Under physiological conditions, Nrf2 binds kelch-like epichlorohydrin-related proteins (Keap1) to form cytosolic complexes. However, when oxidative stress or other stimuli occur, the Keap1-Nrf2 complex is disassembled and Nrf2 translocates to the nucleus, binds to ARE and transcribes target genes such as HO-1, NAD(P)H quinone oxidoreductase 1 and glutathione S-transferase. Curcumin increases production HO-1 via the Nrf2/HO-1 pathway (28,45,46).

Other studies have shown that curcumin inhibits the activation and proliferation of rat PSCs (24,25) *in vitro* model. However, since various factors affect the actual fibrosis process, it is also important to investigate the fibrosis process in an *in vivo* model. Therefore, we aimed to investigate the effects of curcumin on fibrosis in mice *in vivo* and *in vitro* models. Curcumin increased the mRNA and protein levels of HO-1 in PSCs isolated from mice. In addition, curcumin led to Nrf2 activation (translocation from the cytosol to the nucleus) in PSCs and trigonelline, a Nrf2 inhibitor, reduced curcumin-induced HO-1 production. These results indicated that curcumin activated the Nrf2/HO-1 pathway in PSCs. Activation of PSCs by co-treatment with SnPP, a HO-1 inhibitor, was evaluated to determine the effect of curcumin-induced HO-1 on PSCs. In TGF- β -treated PSCs, the mRNA levels of the fibrosis markers Acta2, Tgf- β 1, Col1a1 and Col4a1 increased; curcumin treatment reversed this effect. The inhibitory effect of curcumin on PSC activation was significantly attenuated by blocking HO-1 expression after SnPP treatment. These results suggest that the inhibitory effect of curcumin on PSC activation was mediated by HO-1.

In conclusion, curcumin inhibited the histological damage and pancreatic fibrosis associated with CP. In addition, curcumin treatment of isolated PSCs suppressed the expression of TGF- β -induced fibrosis-associated genes via the Nrf2/HO-1 pathway. Collectively, these results suggested that curcumin may be an effective drug for the treatment of CP.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SJP and GSB conceived the study and designed the experiments. DUK, BK, MJK and DGK performed the experiments. DUK and BK wrote and revised the manuscript. JYO, GRN, YL and JY analyzed data and constructed the figures. SJP and GSB confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All experiments were approved by the Wonkwang University Animal Ethics Committee (approval no. WKU 25-4).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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