

# Lutein inhibits IL-6 expression by inducing PPAR- $\gamma$ activation and SOCS3 expression in cerulein-stimulated pancreatic acinar cells

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**Abstract.** Acute pancreatitis is a severe inflammatory disease of the pancreas. In experimental acute pancreatitis, cerulein induces the expression of interleukin-6 (IL-6) by activating Janus kinase (JAK) 2/signal transducer and activator of transcription (STAT) 3 in pancreatic acinar cells. Ligands of peroxisome proliferator activated receptor- $\gamma$  (PPAR- $\gamma$ ) and suppressor of cytokine signaling (SOCS) 3 inhibit IL-6 expression by suppressing JAK2/STAT3 in cerulein-stimulated pancreatic acinar AR42J cells. Lutein, an oxygenated carotenoid, upregulates and activates PPAR- $\gamma$  to regulate inflammation in a renal injury model. The present study aimed to determine whether lutein activated PPAR- $\gamma$  and induced SOCS3 expression in unstimulated AR42J cells, and whether lutein inhibited activation of JAK2/STAT3 and IL-6 expression via activation of PPAR- $\gamma$  and SOCS3 expression in cerulein-stimulated AR42J cells. The anti-inflammatory mechanism of lutein was determined using reverse transcription-quantitative PCR, western blot analysis and enzyme-linked immunosorbent assay in AR42J cells stimulated with or without cerulein. In another experiment, cells were treated with lutein and the PPAR- $\gamma$  antagonist GW9662 or the PPAR- $\gamma$  agonist troglitazone prior to cerulein stimulation to determine the involvement of PPAR- $\gamma$  activation. The results indicated that lutein increased PPAR- $\gamma$  and SOCS3 levels in unstimulated cells. Cerulein increased phospho-specific forms of JAK2 and STAT3, and mRNA and protein expression of IL-6, but decreased SOCS3 levels in AR42J cells. Cerulein-induced alterations were suppressed by lutein or troglitazone. GW9662 alleviated the inhibitory effect of lutein on JAK2/STAT3 activation and IL-6 expression in cerulein-stimulated cells. In conclusion, lutein inhibited the activation of JAK2/STAT3 and reduced IL-6

levels via PPAR- $\gamma$ -mediated SOCS3 expression in pancreatic acinar cells stimulated with cerulein.

## Introduction

Acute pancreatitis is an acute inflammatory process of the pancreas caused by the intracellular activation of digestive enzymes and autodigestion of the pancreas (1). Destruction of the pancreatic parenchyma induces a rapid inflammatory process at the injury site (2). During the early phase of acute pancreatitis, pancreatic acinar cells produce tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-6, and chemokine monocyte chemoattractant protein-1 (MCP-1). These findings have been observed in pancreatic acinar cells of patients with acute pancreatitis (3). IL-6, a member of the inflammatory cytokine family, is the primary inducer of acute-phase protein responses during all types of injuries, and its level is directly correlated with the severity of the injury. Therefore, the severity of pancreatitis is indicated by the degree and duration of IL-6 elevation (4,5).

In an experimental pancreatitis model, a cholecystokinin (CCK) analog, cerulein, induced pancreatic inflammation similar to human acute pancreatitis. Treatment with high doses of cerulein increases the levels of serum digestive enzymes (amylase and lipase) and cytokines in the pancreas (6,7). Previously, we demonstrated that cerulein induces the activation of Janus kinase (JAK) 2, signal transducer and activator of transcription (STAT) 3, and IL-1 $\beta$  expression, which were suppressed by treatment with the JAK inhibitor AG490 in pancreatic acinar cells. In cerulein-stimulated pancreatitis in rats, AG490 treatment inhibited pancreatic changes, such as edema and inflammation, and reduced serum IL-6 levels (8,9). Therefore, the Jak2/Stat3 pathway may be the upstream signaling pathway for cytokine expression in the pathogenesis of pancreatitis.

The JAK/STAT pathway mediates cytokine signal transduction in the immune system and in other tissues (10). In pancreatic acinar cells, cerulein binds to the G protein-coupled receptor CCK2 and activates the JAK2/STAT3 pathway for cell proliferation (11). Subsequently, JAK/STAT activation leads to the induction of suppressors of cytokine signaling (SOCS) proteins, which suppress cytokine signaling (12). SOCS inhibits JAK activity and negatively regulates immune cell response (13). Our previous study revealed that ligands

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of peroxisome proliferator activated receptor- $\gamma$  (PPAR- $\gamma$ ), such as 15-deoxy- $\Delta$ 12, 14-prostaglandin J2 (15d-PGJ2) and troglitazone, and suppressor of cytokine signaling (SOCS) 3 inhibit IL-6 expression by suppressing JAK2/STAT3 in cerulein-stimulated pancreatic acinar cells (9). Berlato *et al* reported that transfection of SOCS3 into mouse macrophages decreased nitric oxide, TNF- $\alpha$ , and IL-6 levels (14). Therefore, a relationship exists between JAK2/STAT3, PPAR- $\gamma$ , and SOCS3 in cells exposed to cytokines or inflammatory stimuli.

PPAR- $\gamma$ , a nuclear hormone receptor, regulates the metabolism of fatty acids and glucose (15). It also contributes to the inactivation of genes involved in inflammation (16). When PPAR- $\gamma$  is activated by ligands, dimerization with the retinoic acid X receptor (RXR) binds to the peroxisome proliferator response element (PPRE) to induce various target genes (17). Rollins *et al* reported that PPAR- $\gamma$  agonists (15d-PGJ2 or troglitazone) significantly reduced the severity of pancreatitis in mice by reducing serum amylase activity and the levels of pro-inflammatory cytokines, such as IL-6 and TNF- $\alpha$  (18). Furthermore, PPAR- $\gamma$  induces inflammation regulation by SOCS3. Berger *et al* showed that docosahexaenoic acid (DHA)-mediated PPAR- $\gamma$  activation limits Th17 cell differentiation by inducing SOCS3 expression (19). DHA may bind to PPAR- $\gamma$  and transactivate the SOCS3 promoter to prevent the phosphorylation of STAT3 (20). Therefore, SOCS3 might prevent JAK/STAT3 activation and decrease the expression of inflammatory genes.

Lutein is a lipophilic oxygenated carotenoid present in green leafy vegetables, fruits, and egg yolk. Since lutein exerts antioxidant and anti-inflammatory effects, it suppresses oxidative stress-mediated inflammatory diseases such as neurodegenerative disorders, diabetic retinopathy, and colon diseases (21). Dietary lutein supplementation (50 mg/kg body weight) inhibits LPS-induced elevation of splenic levels of IL-1 and PPAR- $\gamma$  in chickens (22). Additionally, lutein protects against vancomycin-induced renal injury by upregulating PPAR- $\gamma$  (23).

In the present study, we investigated whether lutein activates PPAR- $\gamma$  and induces SOCS3 expression, thereby inhibiting cerulein-stimulated activation of JAK2/STAT3 and IL-6 expression in pancreatic acinar AR42J cells. To investigate the involvement of PPAR- $\gamma$  activation in IL-6 expression, cerulein-stimulated cells were treated with the PPAR- $\gamma$  antagonist GW9662 in the presence of lutein or the PPAR- $\gamma$  agonist troglitazone.

## Materials and methods

**Materials.** Lutein, troglitazone, the PPAR- $\gamma$  antagonist GW9662, and cerulein were obtained from Sigma-Aldrich; Merck KGaA. Lutein, troglitazone, and GW9662 were dissolved in DMSO. Cerulein was dissolved in PBS containing 0.1% BSA ( $10^{-4}$  M). For each experiment, the amount of vehicle DMSO was <0.1%.

**Cell line and culture conditions.** Rat pancreatic acinar AR42J cells (pancreatoma, ATCC CRL 1492) were purchased from the American Type Culture Collection (ATCC) and cultured as previously described (9).

**Experimental protocol.** To investigate the effect of lutein on PPAR- $\gamma$  activation and SOCS3 expression in unstimulated cells, AR42J cells ( $8 \times 10^5$  cells/ml/well) were treated with 5  $\mu$ M lutein for 1, 2, or 3 h. Activation of PPAR- $\gamma$  was increased by lutein treatment (peak at 1 h and still elevated at 2 h) in unstimulated cells. The levels of SOCS3 were increased by lutein (elevated at 2 h and peaked at 3 h) in unstimulated cells. Therefore, to determine the effective dose of lutein, the cells were treated with 1, 2, and 5  $\mu$ M lutein for 1 h to assess its effect on PPAR- $\gamma$  expression and for 3 h to measure SOCS3 expression levels.

To determine the effect of lutein on cerulein-stimulated cells, AR42J cells were treated with 5  $\mu$ M lutein for 2 h and then stimulated with cerulein ( $10^{-8}$  M) for 1 h (JAK2/STAT3 activation), 6 h (IL-6 mRNA expression), and 24 h (IL-6 protein expression) based on our previous studies (8,21,24). A 1 h incubation time for cerulein-induced JAK2/STAT3 activation was adapted from our previous study (8,24), while incubation times for IL-6 mRNA and protein levels were adapted from the study by Ahn and Kim (21).

To assess whether PPAR- $\gamma$  and SOCS3 contribute to the inhibitory effect of lutein on cerulein-stimulated IL-6 expression, cells were pretreated with 10  $\mu$ M GW9662 in the presence of 5  $\mu$ M lutein or 40  $\mu$ M troglitazone for 2 h and stimulated with cerulein for 1 h (JAK2/STAT3 activation), 6 h (IL-6 mRNA), and 24 h (IL-6 protein expression).

**Real-time polymerase chain reaction (PCR) analysis.** IL-6 mRNA expression levels were determined using a method described previously (25). The IL-6 (accession number M26745) primers 5'-GCCCTTCAGGAACAGCTATGA-3' (forward primer) and 5'-TGTCACAACATCAGTCCCAAGA-3' (reverse primer) were used to generate a 242 bp PCR product. For  $\beta$ -actin (accession number XM\_032887061.1), the forward primer used was 5'-ACCAACTGGGACGATATGGAG-3' and the reverse primer was 5'-GTCAGGATCTTCATGAGGTAGTC-3', which gives a 353 bp PCR product.  $\beta$ -actin, a reference gene, was amplified in the same reaction.

**Enzyme-linked immunosorbent assay.** IL-6 level in the culture medium was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen; Thermo Fisher Scientific, Inc.) (25).

**Western blot analysis.** Preparation of whole-cell, cytosolic, and nuclear extracts, as well as western blot analysis were performed as previously described (26). Briefly, the extracts (6-40  $\mu$ g protein/per lane) were loaded onto 8-10% SDS polyacrylamide gels and separated by electrophoresis. The following antibodies were used: p-JAK2 (#3771), p-STAT3 (#9131), JAK2 (#3230) (Cell Signaling Technology), STAT3 (sc-483), PPAR- $\gamma$  (sc-7273), aldolase A (sc-12059), histone H3 (sc-10809), SOCS3 (sc-518020), and actin (sc-1615; Santa Cruz Biotechnology). Primary antibodies were detected with horseradish peroxidase-conjugated secondary antibodies and visualized using the enhanced chemiluminescence detection system (Santa Cruz Biotechnology) and BioMax MR film (Kodak) using the enhanced chemiluminescence detection system (Santa Cruz Biotechnology). Actin, aldolase A, and histone H3 served as controls in whole-cell, cytosolic, and

nuclear extracts, respectively. The levels of PPAR- $\gamma$  and SOCS3 in the whole-cell extracts were compared to those of actin. The levels of PPAR- $\gamma$  in the cytosolic and nuclear extracts were compared to those of aldolase A and histone H3, respectively. The phospho-specific forms of JAK2 and STAT3 were compared with those of total JAK2 and STAT3. The intensity of each protein band was quantified using ImageJ software (National Institutes of Health). The data represent the mean  $\pm$  standard error (SE) from three immunoblots. For the relative protein expression, the relative density of the protein band was normalized to actin, aldolase A, histone 3, total JAK2, or total STAT3.

**Statistical analysis.** Values are represented as mean  $\pm$  SE (n=12 per group). Statistical analysis was performed using analysis of variance (ANOVA), followed by individual comparisons using Tukey's post-hoc test. Differences were considered significant at a P-value of  $\leq 0.05$ .

## Results

*Lutein promotes expression and nuclear translocation of PPAR- $\gamma$  and expression of SOCS3 in unstimulated AR42J cells.* First, we determined the effect of lutein on PPAR- $\gamma$  activation and SOCS expression in the unstimulated cells. Cells were treated with 5  $\mu$ M lutein for 1, 2, and 3 h. Fig. 1A shows that the PPAR- $\gamma$  and SOCS3 levels increased at 1 and 3 h, respectively. Therefore, for the dose experiment, lutein (1, 2, and 5  $\mu$ M) treatment was conducted for 1 h to assess PPAR- $\gamma$  expression levels, and for 3 h to measure SOCS3 expression levels (Fig. 1B and C). Treatment with 5  $\mu$ M lutein resulted in a maximum increase in the expression levels of both PPAR- $\gamma$  and SOCS3 in unstimulated cells. Thus, for the next experiment on PPAR- $\gamma$  activation, cells were treated with 5  $\mu$ M lutein.

To observe the nuclear translocation of PPAR- $\gamma$ , the cells were treated with 5  $\mu$ M lutein for 1 h, and PPAR- $\gamma$  protein levels were determined by western blot analysis (Fig. 1D). PPAR- $\gamma$  expression was observed at low levels in both cytosolic and nuclear extracts of the untreated cells (lutein, -) and lutein treatment increased PPAR- $\gamma$  expression in both cytosolic and nuclear extracts. Aldolase A and histone H3, the indices of cytosolic and nuclear extracts, were not altered by lutein treatment.

*Lutein suppresses activation of JAK2/STAT3 and expression of IL-6 but increases SOCS3 expression level in AR42J cells stimulated with cerulein.* Fig. 2A and B show that cerulein increased IL-6 mRNA and protein levels, which were reversed by lutein. Without cerulein stimulation, both mRNA and protein levels of IL-6 were reduced by lutein treatment.

To investigate the underlying mechanism, the activation of JAK2/STAT3 was assessed in cells treated with or without lutein. Cerulein increased phospho-specific forms of JAK2/STAT3, which were decreased by lutein treatment. The total levels of JAK2/STAT3 were unchanged in cells stimulated with cerulein (Fig. 2C). The expression level of SOCS3, a negative feedback regulator of JAK2/STAT3, was reduced by cerulein, which was inhibited by lutein in AR42J cells. Lutein significantly increased SOCS3 expression level in the unstimulated cells. These results demonstrated that lutein

inhibits JAK2/STAT3 activation and IL-6 expression, which may be mediated by SOCS3 expression in cerulein-stimulated AR42J cells.

*PPAR- $\gamma$  antagonist GW9662 abolishes the effect of lutein on induction of SOCS3 expression and inhibition of IL-6 expression in AR42J cells treated with cerulein.* GW9662 reversed the inhibitory effects of lutein on cerulein-induced expression of IL-6 (Fig. 3A and B). These results demonstrate that lutein inhibits cerulein-induced IL-6 expression by activating PPAR- $\gamma$ . In addition, GW9662 suppressed the effect of lutein on SOCS3 expression (Fig. 3C). Thus, lutein may act as a PPAR- $\gamma$  agonist to activate PPAR- $\gamma$  and increase SOCS3 levels in cerulein-stimulated cells.

*PPAR- $\gamma$  agonist troglitazone inhibits IL-6 expression, but increases SOCS3 expression level in AR42J cells stimulated with cerulein.* Troglitazone reduced mRNA and protein levels of IL-6 in AR42J cells stimulated with cerulein (Fig. 4A and B). The cerulein-induced increase in JAK2 and STAT3 phosphorylation was inhibited by troglitazone (Fig. 4C). In addition, troglitazone markedly increased SOCS3 expression level in the unstimulated cells. The cerulein-induced decrease in SOCS3 levels was inhibited by troglitazone treatment. These results demonstrate that PPAR- $\gamma$  activation by troglitazone inhibits cerulein-induced IL-6 expression and JAK2/STAT3 activation by upregulating SOCS3 expression.

## Discussion

In the present study, lutein attenuated IL-6 expression in cerulein-treated pancreatic acinar cells by inducing PPAR- $\gamma$  activation and SOCS3 expression. We also investigated the relationship between the PPAR- $\gamma$  ligand and SOCS3 expression using the PPAR- $\gamma$  antagonist GW9662. Our previous studies demonstrated that PPAR- $\gamma$  ligands, such as 15d-PGJ2 and troglitazone, decreased cerulein-induced expression of IL-6 and TGF- $\beta$  by upregulating SOCS3 in pancreatic acinar cells (8,9). GW9662 reversed the inhibitory effect of DHA on IL-6 expression in cerulein-treated AR42J cells (27). Rollins *et al* demonstrated that the PPAR- $\gamma$  agonists 15d-PGJ2 and troglitazone reduced the severity of pancreatitis by reducing serum amylase activity and histological damage (leukocyte infiltration and vacuolization) in the pancreas (18). 15d-PGJ2 and ciglitazone prevented inflammation by increasing PPAR- $\gamma$  target genes, such as antioxidant enzymes (copper/zinc superoxide dismutase and catalase), and showed neuroprotective effects (28).

Activation of STAT proteins is tightly regulated by SOCS proteins. SOCS proteins are direct targets of STATs that inhibit JAK/STAT activation, and SOCS3 regulates cellular processes, including growth, apoptosis, and inflammatory gene expression (29). SOCS3 deficiency induces embryonic lethality through the activation of STAT3 and mitogen-activated protein kinases (30). Cerulein activates NADPH oxidase; consequently, large amounts of ROS activate the JAK2/STAT3 pathway and induce IL-6 expression in pancreatic acinar cells (9,24). Carballo *et al* (31) demonstrated that hydrogen peroxide promotes the nuclear translocation of STAT3 in human lymphocytes. This study revealed oxidative stress as a

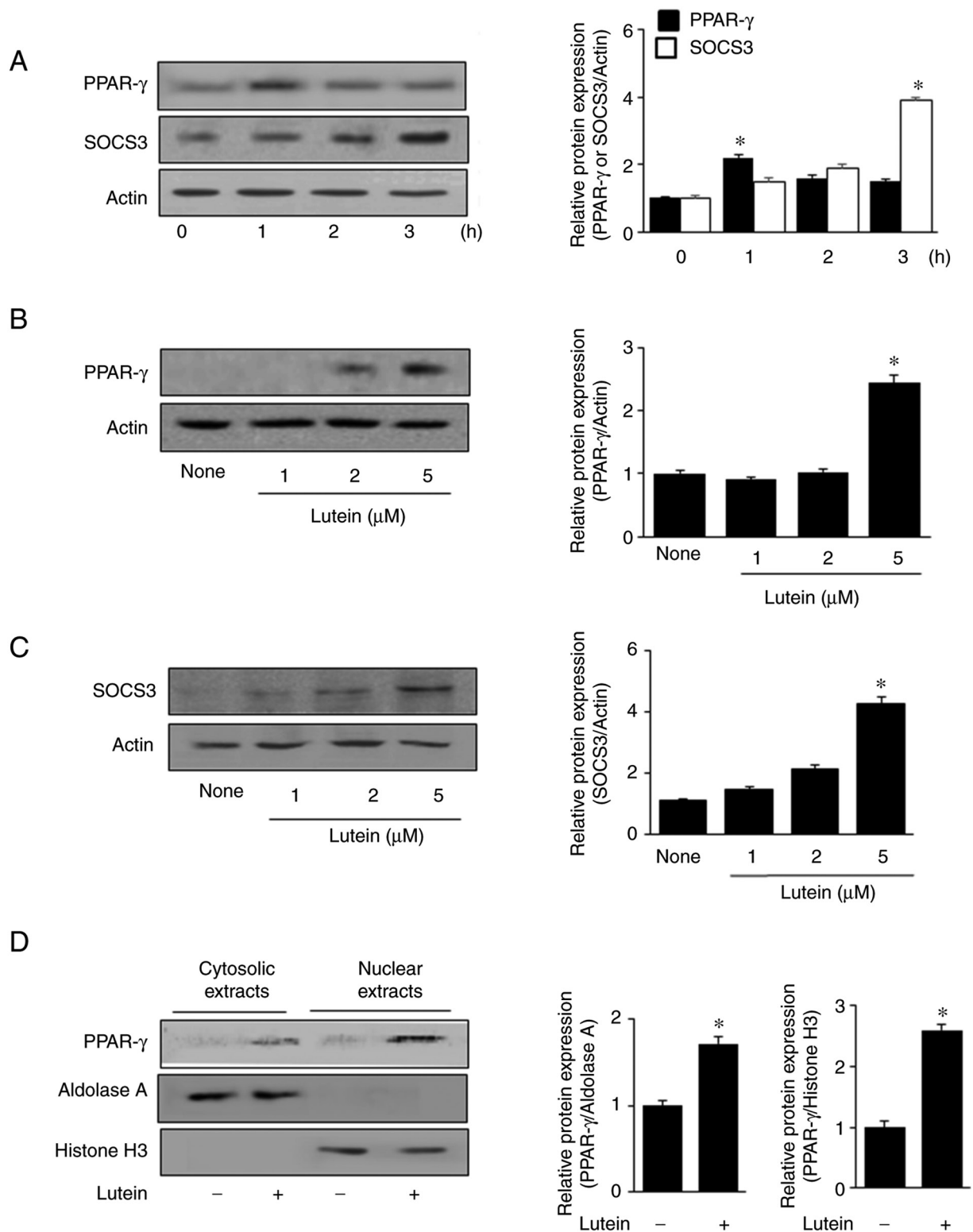


Figure 1. Lutein promotes PPAR- $\gamma$  expression and SOCS3 expression in unstimulated AR42J cells. (A) Cells were cultured with 5  $\mu$ M lutein for the indicated time periods. (B) Cells were cultured with the indicated concentrations of lutein for (B) 1 h and (C) 3 h. (A-C) Western blot analysis was used for protein levels of PPAR- $\gamma$  or SOCS3. Actin served as the loading control (left panel). The densitometry analysis for the ratio of PPAR- $\gamma$ /actin or SOCS3/actin represent the mean  $\pm$  SE from three immunoblots. (D) Cells were cultured with 5  $\mu$ M lutein for 1 h. Protein levels of PPAR- $\gamma$  in cytosolic or nuclear extracts were examined by western blot analysis. Aldolase A and histone H3 served as cytosolic and nuclear markers (left panel). For the relative protein expression, the densitometry analysis for the ratio of PPAR- $\gamma$ /aldolase A or PPAR- $\gamma$ /histone H3 represent the mean  $\pm$  SE from three immunoblots (right panel). 'None' represents untreated cells (Lutein, -). \* $P < 0.05$  vs. the corresponding 0 h or 'None'. PPAR- $\gamma$ , peroxisome proliferator activated receptor- $\gamma$ ; SOCS3, suppressor of cytokine signaling.

transcription factor of STAT3. Therefore, cerulein-induced ROS production may activate JAK/STAT, which may induce SOCS3 expression as a negative feedback loop. Our previous study

showed that cerulein increased SOCS3 expression, but this induction of SOCS3 was not sufficient to prevent IL-6 expression in pancreatic acinar cells (9). PPAR- $\gamma$  agonists 15d-PGJ2

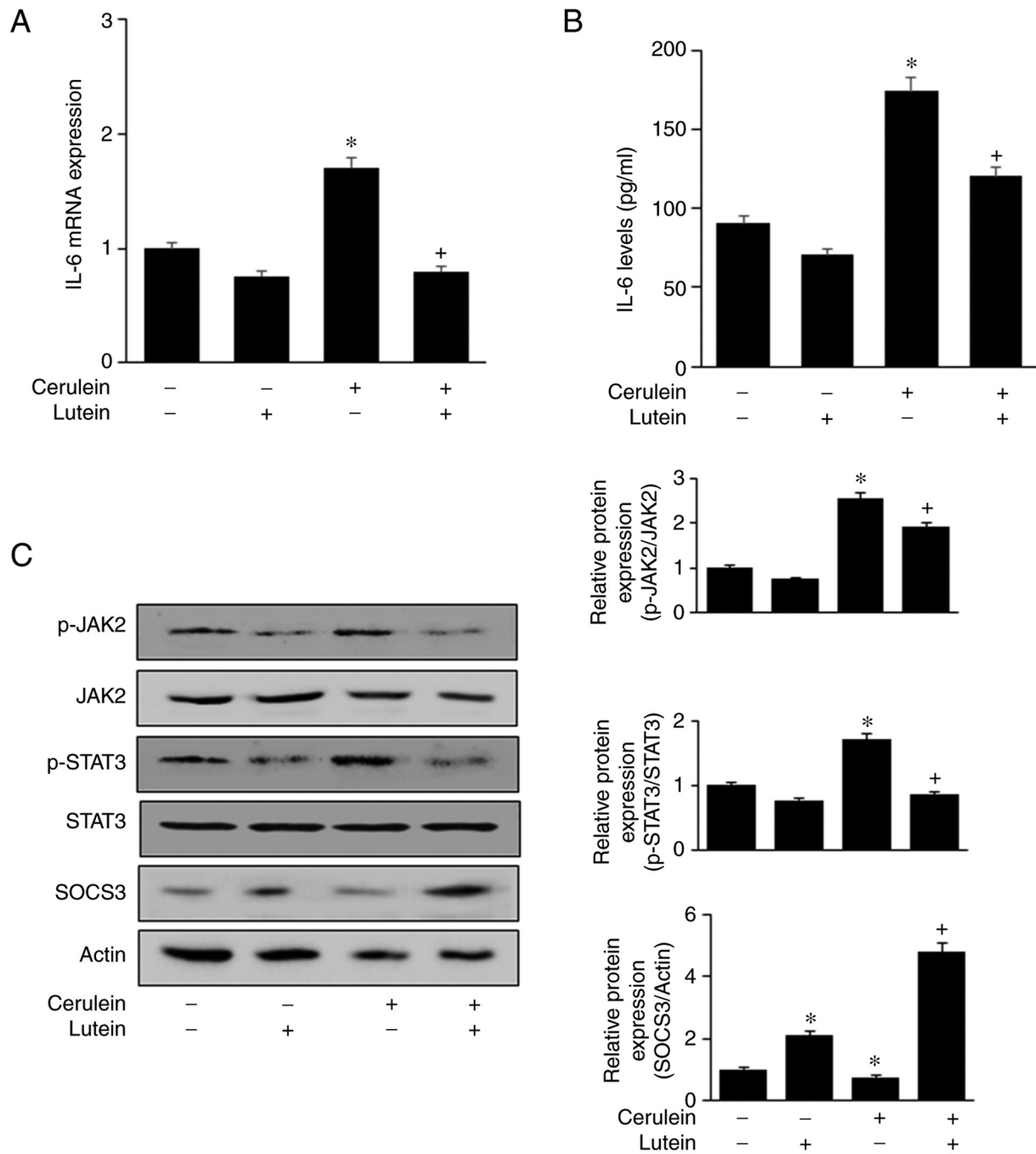


Figure 2. Lutein suppresses activation of JAK2/STAT3 and expression of IL-6, but induces SOCS3 expression in AR42J cells stimulated with cerulein. Cells were cultured with 5  $\mu$ M lutein for 2 h and stimulated in the presence or absence of cerulein ( $10^{-8}$  M) for (A) 6 h or (B) 24 h. (A) mRNA level of IL-6 was determined using reverse transcription-quantitative PCR and normalized to the level of  $\beta$ -actin. (B) IL-6 levels in the medium were determined by ELISA (n=12 per each group). (C) Cells were cultured with 5  $\mu$ M lutein for 2 h and then treated with cerulein ( $10^{-8}$  M) for 1 h. Total and phospho-specific forms of JAK2 and STAT3, and level of SOCS3 in whole-cell extracts were determined by western blot analysis (left panel). For the relative protein expression, the densitometry analysis for the ratio of p-JAK2/JAK2, p-STAT3/STAT3 and SOCS3/actin represent the mean  $\pm$  SE from three immunoblots (right panel). \*P<0.05 vs. the unstimulated and untreated cells (Cerulein<sup>-</sup>, Lutein<sup>-</sup>); †P<0.05 vs. cerulein-treated cells without lutein treatment (Cerulein<sup>+</sup>, Lutein<sup>-</sup>). JAK2, Janus kinase; STAT, signal transducer and activator of transcription; IL, interleukin; SOCS3, suppressor of cytokine signaling; p-, phosphorylated.

and troglitazone increased SOCS3 levels and prevented IL-6 expression in cerulein-stimulated cells (9). Therefore, nutrients that act as PPAR- $\gamma$  agonists to induce SOCS3 expression, may be beneficial in preventing acute pancreatitis.

Gallmeier *et al* showed that acinar cells are the main source of STATs in the pancreas (32). In rat pancreatic acinar cells, increased STAT3 and SOCS3 mRNA levels are observed in response to the inflammatory mediator TNF- $\alpha$  (33). STAT3

regulates the expression of IL-6 during starvation-induced autophagy in cancer cells (34). In cultured vascular cells, SOCS overexpression prevents the proliferation of vascular smooth muscle cells and monocytes, whereas SOCS3 inhibition leads to an increase in the magnitude of cytokine response (35). These studies support the present finding that JAK2/STAT3 activation mediates IL-6 production in cerulein-stimulated AR42J cells.

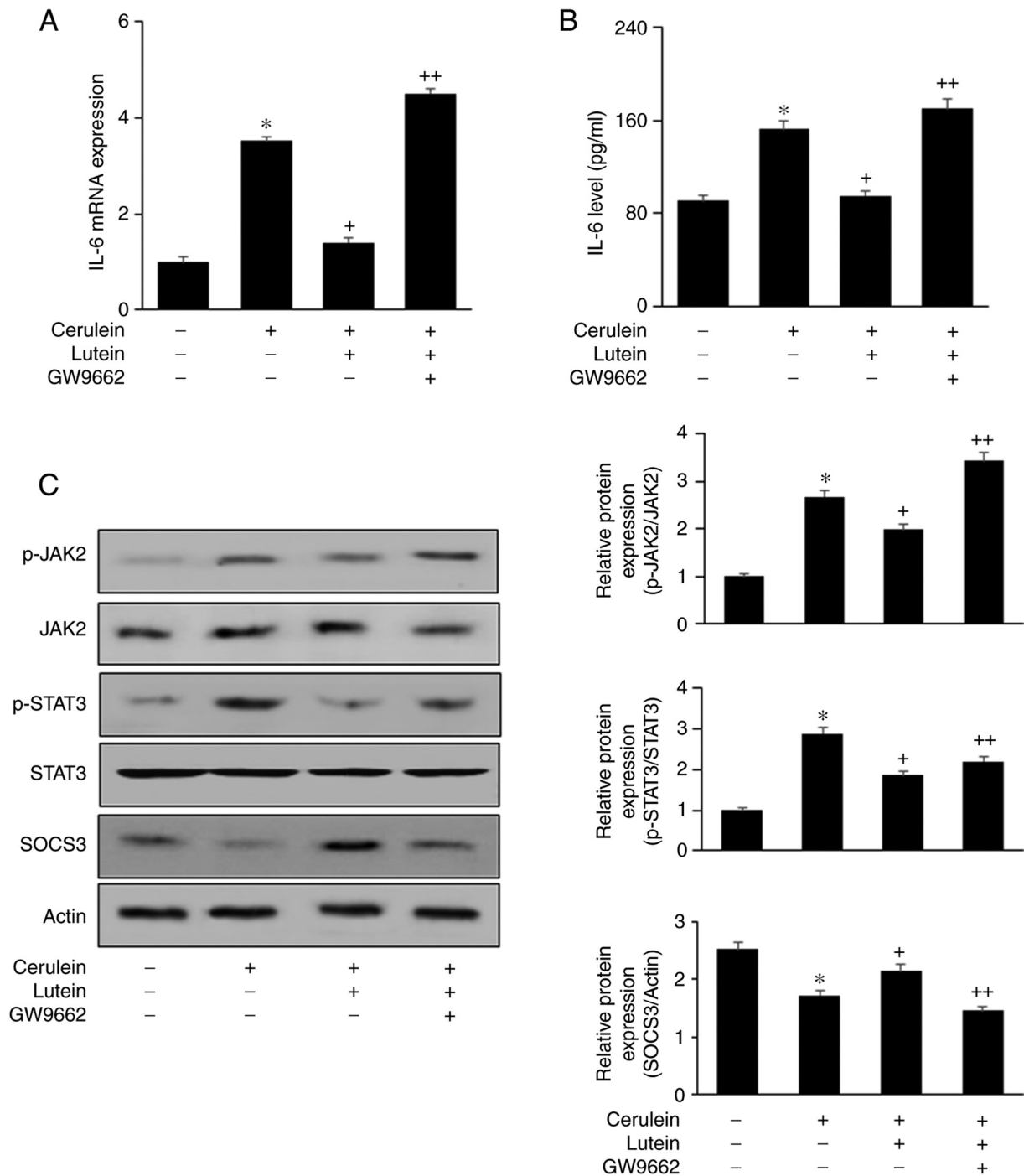


Figure 3. GW9662, a PPAR- $\gamma$  antagonist, reverses the effect of lutein on induction of SOCS3 expression and inhibition of IL-6 expression in AR42J cells stimulated with cerulein. Cells were co-treated with lutein (5  $\mu$ M) and GW9662 (1  $\mu$ M) for 2 h, and then cultured with cerulein for (A) 6 h, (B) 24 h or (C) 1 h. (A) mRNA expression level of IL-6 was determined using reverse transcription PCR and normalized to the expression level of  $\beta$ -actin. (B) IL-6 levels in the cell culture media were determined by ELISA (n=12 per each group). (C) Total and phospho-specific forms of JAK2 and STAT3, and level of SOCS3 in whole-cell extracts were determined using western blot analysis (left panel). For the relative protein expression, the densitometry analysis for the ratio of p-JAK2/JAK2, p-STAT3/STAT3 and SOCS3/actin represent the mean  $\pm$  SE from three immunoblots (right panel). \*P<0.05 vs. the unstimulated and untreated cells (Cerulein<sup>-</sup>, Lutein<sup>-</sup>, GW9662<sup>-</sup>); \*P<0.05 vs. Cerulein-treated cells without any treatment (Cerulein<sup>+</sup>, Lutein<sup>-</sup>, GW9662<sup>-</sup>). \*\*P<0.05 vs. Cerulein and lutein-treated cells without GW9662 treatment (Cerulein<sup>+</sup>, Lutein<sup>+</sup>, GW9662<sup>-</sup>). PPAR- $\gamma$ , peroxisome proliferator-activated receptor- $\gamma$ ; SOCS3, suppressor of cytokine signaling; IL, interleukin; JAK2, Janus kinase; STAT, signal transducer and activator of transcription; p-, phosphorylated.

Lutein modulates proinflammatory mediators, such as PPAR, which affects inflammatory signaling pathways (36). PPAR- $\gamma$  has been implicated in the regulation of glucose, lipid homeostasis, cell differentiation, and apoptosis (37). It is a sensor of lipophilic molecules that causes structural changes in the PPAR- $\gamma$  receptor, which activates this receptor. In its

active conformation, PPAR- $\gamma$  induces regulatory actions in inflammation (38). Dietary lutein induces PPAR expression and inhibits inflammation in chicken immune tissues (22). These studies support our present findings that lutein inhibits cerulein-induced IL-6 expression through PPAR- $\gamma$  activation and SOCS3 induction in pancreatic acinar cells.

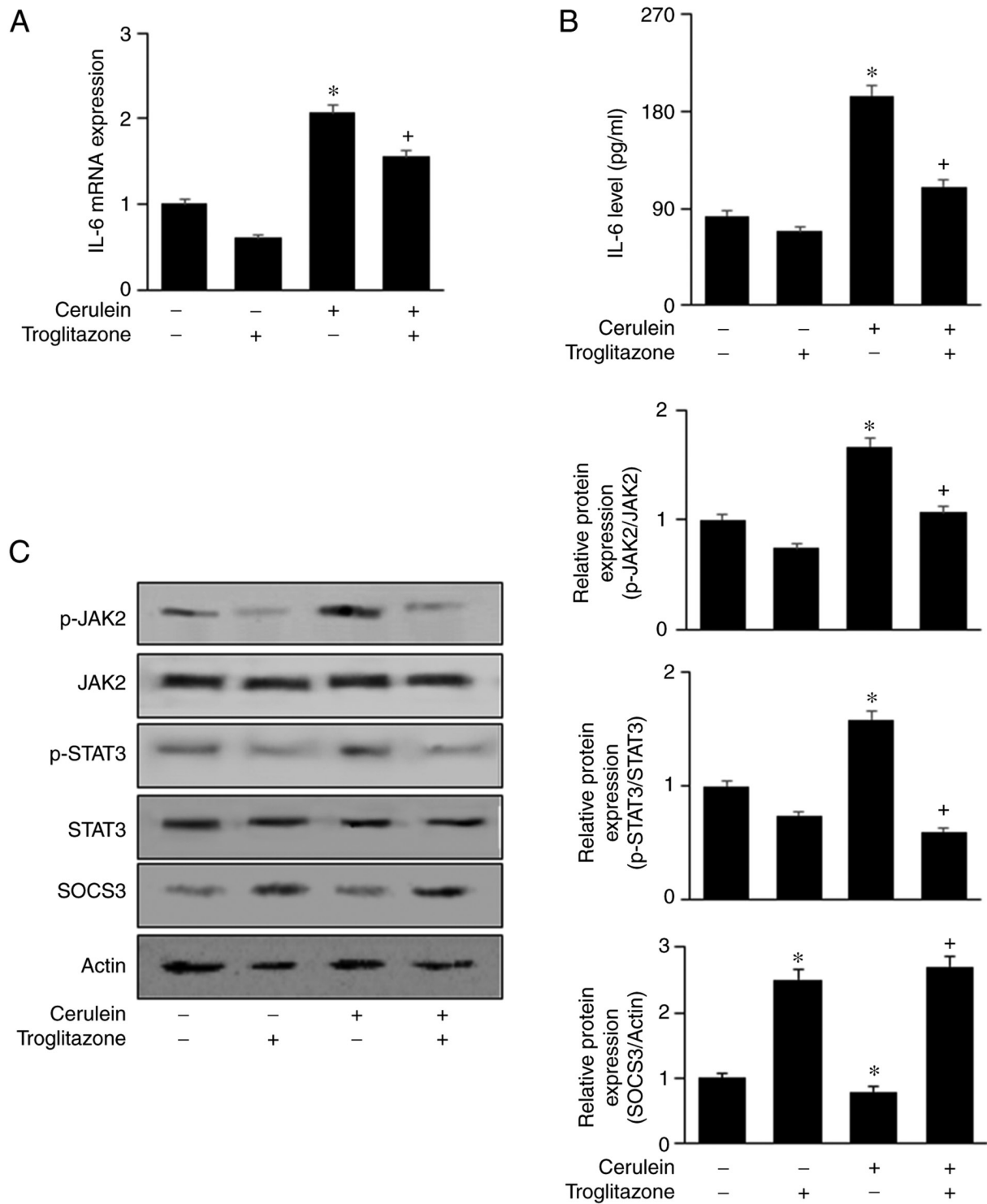


Figure 4. PPAR- $\gamma$  agonist troglitazone inhibits IL-6 expression but induces SOCS3 expression in AR42J cells stimulated with cerulein. Cells were treated with 40  $\mu$ M troglitazone for 2 h and cultured with cerulein for (A) 6 h, (B) 24 h or (C) 1 h. (A) IL-6 mRNA level was determined by reverse transcription-quantitative PCR and normalized to the level of  $\beta$ -actin. (B) IL-6 levels in the medium were measured by ELISA (n=12 per each group). (C) Levels of total and phospho-specific forms of JAK2, STAT3, and SOCS3 in whole-cell extracts were determined using western blot analysis (left panel). For the relative protein expression, the densitometry analysis for the ratio of p-JAK2/JAK2, p-STAT3/STAT3 and SOCS3/actin represent mean  $\pm$  SE from three immunoblots (right panel). \*P<0.05 vs. the unstimulated and untreated cells (cerulein<sup>-</sup>, Troglitazone<sup>-</sup>); +P<0.05 vs. cerulein-treated cells without troglitazone treatment (cerulein<sup>+</sup>, Troglitazone<sup>-</sup>). SOCS3, suppressor of cytokine signaling; IL, interleukin; JAK2, Janus kinase; STAT, signal transducer and activator of transcription; p-, phosphorylated.

For the first time, we show that the expression of PPAR- $\gamma$  is stimulated by lutein, which increases the expression of SOCS3 in pancreatic acinar cells. Since this antioxidant mechanism of lutein has not been reported in any cells or tissues, the results of this study may increase the use of

lutein for preventing and treating oxidative stress-associated diseases in various tissues. Further studies are needed to determine whether lutein increases SOCS3 expression via PPAR- $\gamma$  activation in pancreatic tissues of animals with acute pancreatitis.

The limitation of the present study is that only one cell line was used to determine the antioxidant mechanism of lutein and its efficacy in preventing acute pancreatitis progression. Since acute pancreatitis is a serious state of pancreatitis, the demonstrated inhibitory effect of lutein on cerulein-stimulated IL-6 expression in pancreatic acinar cells does not confirm the protective effect of lutein on the development and progression of acute pancreatitis. Further studies on the effects of lutein on systematic and local events in acute pancreatitis development and progression should be performed using *in vivo* experiments. Thus, it cannot be concluded that lutein reduces the inflammatory response in acute pancreatitis.

The novel finding of the present study was that lutein exhibits antioxidant properties via PPAR- $\gamma$  activation and SOCS3 induction in pancreatic acinar cells. Therefore, lutein inhibited cerulein-mediated JAK2/STAT3 activation and IL-6 expression in pancreatic acinar cells. In conclusion, lutein-induced PPAR- $\gamma$  activation and SOCS3 expression may underlie the inhibitory effect of lutein on cerulein-induced IL-6 expression in pancreatic acinar AR42J cells.

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### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

### Authors' contributions

HK conceived and designed the experiments. JW assisted in the experimental design. YJA performed the experiments. YJA and JW analyzed the data. YJA and JW confirmed the authenticity of all raw data. YJA wrote the manuscript. HK reviewed and edited the manuscript. All the authors have read and approved the final manuscript.

### Ethics approval and consent to participate

Not applicable.

### Patient consent for publication

Not applicable.

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### Competing interests

The authors declare that they have no competing interests.

### References

1. Leach SD, Gorelick FS and Modlin IM: New perspectives on acute pancreatitis. *Scand J Gastroenterol* 27 (Suppl 192): S29-S38, 1992.
2. Banks P: Predictors of severity in acute pancreatitis. *Pancreas* 6 (Suppl 1): S7-S12, 1991.
3. Gu H, Werner J, Bergmann F, Whitcomb DC, Büchler MW and Fortunato F: Necro-inflammatory response of pancreatic acinar cells in the pathogenesis of acute alcoholic pancreatitis. *Cell Death Dis* 4: e816, 2013.
4. Biffi WL, Moore EE, Moore FA and Peterson VM: Interleukin-6 in the injured patients: Marker of injury or mediator of inflammation. *Ann Surg* 224: 647-664, 1996.
5. Kishimoto T, Akira S, Narazaki M and Taga T: Interleukin-6 family of cytokines and gp130. *Blood* 86: 1243-1254, 1995.
6. Hyun JJ and Lee HS: Experimental models of pancreatitis. *Clin Endosc* 47: 212-216, 2014.
7. Kim H: Cerulein pancreatitis: Oxidative stress inflammation and apoptosis. *Gut Liver* 2: 74-80, 2008.
8. Yu JH, Kim KH and Kim H: Suppression of IL-1 $\beta$  expression by the Jak 2 inhibitor AG490 in cerulein-stimulated pancreatic acinar cells. *Biochem Pharmacol* 72: 1555-1562, 2006.
9. Yu JH, Kim KH and Kim H: SOCS 3 and PPAR- $\gamma$  ligands inhibit the expression of IL-6 and TGF- $\beta$ 1 by regulating JAK2/STAT3 signaling in pancreas. *Int J Biochem Cell Biol* 40: 677-688, 2008.
10. Watowich SS, Wu H, Socolovsky M, Klingmuller U, Constantinescu SN and Lodish HF: Cytokine receptor signal transduction and the control of hematopoietic cell development. *Annu Rev Cell Dev Biol* 12: 91-128, 1996.
11. Ferrand A, Kowalski-Chauvel A, Bertrand C, Escricret C, Mathieu A, Portolan G, Pradayrol L, Fourmy D, Dufresne M and Seva C: A novel mechanism for JAK2 activation by a G protein-coupled receptor, the CCK2R: Implication of this signaling pathway in pancreatic tumor models. *J Biol Chem* 280: 10710-10715, 2005.
12. Fenner JE, Starr R, Cornish AL, Zhang JG, Metcalf D, Schreiber RD, Sheehan K, Hilton DJ, Alexander WS and Hertzog PJ: Suppressor of cytokine signaling 1 regulates the immune response to infection by a unique inhibition of type I interferon activity. *Nature Immunol* 7: 33-39, 2005.
13. Hilton DJ, Richardson RT, Alexander WS, Viney EM, Willson TA, Sprigg NS, Starr R, Nicholson SE, Metcalf D and Nicola NA: Twenty proteins containing a C-terminal SOCS box form five structural classes. *Proc Nat Acad Sci USA* 95: 114-119, 1998.
14. Berlato C, Cassatella MA, Kinjyo I, Gatto L, Yoshimura A and Bazzoni F: Involvement of suppressor of cytokine signaling-3 as a mediator of the inhibitory effects of IL-10 on lipopolysaccharide-induced macrophage activation. *J Immunol* 168: 6404-6411, 2002.
15. Lazar MA: PPAR  $\gamma$ , 10 years later. *Biochimie* 87: 9-13, 2005.
16. Barish GD, Downes M, Alaynick WA, Yu RT, Ocampo CB, Bookout AL, Mangelsdorf DJ and Evans RM: Nuclear receptor atlas: Macrophage activation. *Mol Endocrinol* 19: 2466-2477, 2005.
17. Zhao W, Shi G, Gu H and Nguyen BN: Role of PPAR $\gamma$  in the nutritional and pharmacological actions of carotenoids. *Res Report Biochem* 6: 13-24, 2016.
18. Rollins MD, Sudarshan S, Firpo MA, Etherington BH, Hart BJ, Jackson HH, Jackson JD, Emerson LL, Yang DT, Mulvihill SJ and Glasgow RE: Anti-inflammatory effects of PPAR- $\gamma$  agonists directly correlate with PPAR- $\gamma$  expression during acute pancreatitis. *J Gastrointest Surg* 10: 1120-1130, 2006.
19. Berger H, Végran F, Chikh M, Gilardi F, Ladoire S, Bugaut H, Mignot G, Chalmin F, Bruchard M, Derangère V, *et al.*: SOCS3 transactivation by PPAR $\gamma$  prevents IL-17-driven cancer growth. *Cancer Res* 73: 3578-3590, 2013.
20. Végran F, Berger H, Ghiringhelli F and Apetoh L: Socs3 induction by PPAR $\gamma$  restrains cancer-promoting inflammation. *Cell Cycle* 12: 2157-2158, 2013.



21. Ahn YJ and Kim H: Lutein as a modulator of oxidative stress-mediated inflammatory diseases. *Antioxidants (Basel)* 10: 1448, 2021.
22. Selvaraj RK, Shanmugasundaram R and Klasing KC: Effects of dietary lutein and PUFA on PPAR and RXR isomer expression in chickens during an inflammatory response. *Comp Biochem Physiol A Mol Integr Physiol* 157: 198-203, 2010.
23. Emeka PM, Rasool ST, Morsy MA, Islam MIH and Chohan MS: Protective effects of lutein against vancomycin-induced acute renal injury in mice via upregulation of peroxisome proliferator-activated receptor gamma/nuclear factor erythroid 2-related factor 2 and inhibition nuclear factor-kappaB/caspase 3. *Kor J Physiol Pharmacol* 25: 321-331, 2021.
24. Yu JH, Kim KH and Kim H: Role of NADPH oxidase and calcium in cerulein-induced apoptosis: Involvement of apoptosis-inducing factor. *Ann NY Acad Sci* 1090: 292-297, 2006.
25. Kwak MS, Lim JW and Kim H: Astaxanthin inhibits interleukin-6 expression in cerulein/resistin-stimulated pancreatic acinar cells. *Med Inflamm* 2021: 5587297, 2021.
26. Lim JW, Kim KH and Kim H: NF-kappaB p65 regulates nuclear translocation of Ku70 via degradation of heat shock cognate protein 70 in pancreatic acinar AR42J cells. *Int J Biochem Cell Biol* 40: 2065-2077, 2008.
27. Song EA, Lim JW and Kim H: Docosahexaenoic acid inhibits IL-6 expression via PPAR $\gamma$ -mediated expression of catalase in cerulein-stimulated pancreatic acinar cells. *Int J Biochem Cell Biol* 88: 60-68, 2017.
28. Kapadia R, Yi JH and Vemuganti R: Mechanisms of anti-inflammatory and neuroprotective actions of PPAR-gamma agonists. *Front Biosci* 13: 1813-1826, 2008.
29. Carow B and Rottenberg ME: SOCS3, a major regulator of infection and inflammation. *Front Immunol* 5: 58, 2014.
30. Roberts AW, Robb L, Rakar S, Hartley L, Cluse L, Nicola NA, Metcalf D, Hilton DJ and Alexander WS: Placental defects and embryonic lethality in mice lacking suppressor of cytokine signaling 3. *Proc Nat Acad Sci USA* 98: 9324-9329, 2001.
31. Carballo M, Conde M, El Bekay R, Martín-Nieto J, Camacho MJ, Monteseirín J, Conde J, Bedoya FJ and Sobrino F: Oxidative stress triggers STAT3 tyrosine phosphorylation and nuclear translocation in human lymphocytes. *J Biol Chem* 274: 17580-17586, 1999.
32. Gallmeier E, Schäfer C, Moubarak P, Tietz A, Plössl I, Huss R, Göke B and Wagner ACC: JAK and STAT proteins are expressed and activated by IFN-gamma in rat pancreatic acinar cells. *J Cell Physiol* 203: 209-216, 2005.
33. Vona-Davis LC, Frankenberry KA, Waheed U, Peterson E and McFadden DW: Expression of STAT3 and SOCS3 in pancreatic acinar cells. *J Surg Res* 127: 14-20, 2005.
34. Yoon S, Woo SU, Kang JH, Kim K, Kwon MH, Park S, Shin HJ, Gwak HS and Chwae YJ: STAT3 transcriptional factor activated by reactive oxygen species induces IL6 in starvation-induced autophagy of cancer cells. *Autophagy* 6: 1125-1138, 2010.
35. Ortiz-Muñoz G, Martín-Ventura JL, Hernandez-Vargas P, Mallavia B, Lopez-Parra V, Lopez-Franco O, Muñoz-García B, Fernandez-Vizarra P, Ortega L, Egido J and Gomez-Guerrero C: Suppressors of cytokine signaling modulate JAK/STAT-mediated cell responses during atherosclerosis. *Arterioscler Thromb Vasc Biol* 29: 525-531, 2009.
36. Kim JE, Clark RM, Park Y, Lee J and Fernandez ML: Lutein decreases oxidative stress and inflammation in liver and eyes of guinea pigs fed a hypercholesterolemic diet. *Nutr Res Pract* 6: 113-119, 2012.
37. Ma Y, Shi M, Wang Y and Liu J: PPAR $\gamma$  and its agonists in chronic kidney disease. *Int J Nephrol* 2020: 2917474, 2020.
38. Desvergne B and Wahli W: Peroxisome proliferator-activated receptors: Nuclear control of metabolism. *Endocr Rev* 20: 649-688, 1999.



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