

# Effects and mechanism of the etanercept on pancreatic encephalopathy

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**Abstract.** Pancreatic encephalopathy (PE) is a common fatal complication of acute pancreatitis (AP). Proinflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-6 are generated during AP, and act synergistically to promote PE and multisystem failure. Caerulein-induced AP provides a convenient model to explore the role of proinflammatory cytokines in PE. The aim of the present study was to examine the effect of the TNF- $\alpha$  inhibitor etanercept in PE models and elucidate the regulatory mechanisms. To model PE *in vitro*, rat hippocampal H19-7/IGF-IR neuronal cells were treated with 10 nmol/ml caerulein alone or in combination with etanercept (1, 10 or 100  $\mu$ mol/ml). To model PE *in vivo*, rats were injected with 50  $\mu$ g/kg caerulein alone or combined with 10 mg/kg etanercept. At 6 h after administration, it was noted that etanercept downregulated expression of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 by negatively regulating NF- $\kappa$ B (a master regulator of cytokine expression) signaling, and prevented the accumulation of reactive oxygen species. Conversely, etanercept promoted the expression of the neurotrophic and anti-inflammatory hypoxia-inducible factor 1  $\alpha$  (HIF-1 $\alpha$ ). In rat hippocampus, etanercept also reduced the levels of TNF- $\alpha$ , IL-1 $\beta$  and IL-6, upregulated HIF-1 $\alpha$  expression and inhibited the inflammatory response to reduce edema and neural necrosis. Together, these data suggested that etanercept could attenuate caerulein-induced PE, at least in part via suppression of NF- $\kappa$ B signaling and alleviation of oxidative stress.

## Introduction

Pancreatic encephalopathy (PE) is a potentially fatal neurological syndrome resulting from acute pancreatic disease, with typical symptoms, including inflammatory or autoimmune reactions, idiopathic nervous system lesions and a neurovegetative state (1). PE is an uncommon complication of severe acute pancreatitis (SAP). Neurological signs can have an acute onset, with convulsions, amaurosis, paresis and dysarthria, or there can be a progressive onset with behavioral changes, psychomotor agitation, space and time disorientation, visual or auditory hallucinations, and affected consciousness that can lead to coma (2). Important elements involved in the complicated evolution of SAP include cell factors, including cytokines such as tumor necrosis factor (TNF)- $\alpha$ , and interleukin (IL)-1 $\beta$ , -6 and -10 (3). In pancreatitis, granulocytes, monocytes and lymphocytes are recruited by local inflammatory processes caused by the release of proinflammatory and anti-inflammatory cytokines and chemokines (4). During pancreatic injury, atrophic acinar cells activate macrophages and granulocytes to release several inflammatory factors, such as IL-1, IL-6 and TNF- $\alpha$  (5). These proinflammatory factors can activate pancreatic stellate cells to accelerate the development of pancreatitis (6). SAP complications, including respiratory insufficiency and hypoxia, can cause abnormal metabolism and cerebral edema (7). The above factors, as part of the multiple organ failure that can occur during PE, lead to a potentially fatal neurological syndrome. The neurological lesions associated with PE probably result from oxidative stress, cytokine storm and enzyme-derived damage (8), but the detailed pathophysiology mechanisms remain to be elucidated.

Adler *et al* (9) found that caerulein can cause severe pancreatitis during pancreatic necrosis. Caerulein, a molecular ortholog of the intestinal hormone cholecystokinin, is derived from the skin of *Litorea caerulea* (10). Caerulein promotes the procession of PE via the disruption of collagen and causes pancreatic fibrosis, initiating edema and exposing neurons to peripheral proinflammatory factors, including TNF- $\alpha$  and IL-1 $\beta$ , which in turn trigger local neuroinflammation, neurodegeneration and demyelination (11). Caerulein also leads to inflammatory cell infiltration, pancreatic edema and acinar

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cell vacuolization that are comparable to acute pancreatitis in humans. Indeed, caerulein exposure is often used to simulate PE *in vitro* or *in vivo* to explore the pathogenic mechanisms of PE (12).

The NF- $\kappa$ B family are a group of structurally related transcription factors that consist of various combinations of NF- $\kappa$ B and Rel proteins (13). NF- $\kappa$ B signaling regulates the expression and release of proinflammatory factors such as TNF- $\alpha$ , IL-1 $\beta$  and IL-6 (14). Transcriptional activity is modulated by the I $\kappa$ B kinase (IKK) complex that regulates the transcriptional activity of NF- $\kappa$ B, as well as I $\kappa$ B $\alpha$ -inhibited NF- $\kappa$ B phosphorylation (15). The IKK subunit IKK $\beta$  is a critical regulator of inflammation via the TNF-induced I $\kappa$ B phosphorylation/degradation pathway and so may regulate the neuroinflammatory response and ensuing nerve cell damage during PE.

Soluble TNF receptors (TNFRs) generated from the cleavage of the TNFR extracellular domain can be used to inhibit TNF- $\alpha$  signaling during inflammatory responses (16). Etanercept is a 934-amino acid (~150 kDa) recombinant dimeric fusion protein consisting of human immunoglobulin G linked via Fc (the constant region) and the extracellular ligand-binding domains of human soluble TNFR2 (17). The dimeric structure of etanercept increases the TNF-binding affinity ~50-fold, which can enhance TNF-neutralizing capacity ~1,000-fold over monomeric TNFR2 (18,19).

During acute pancreatitis (AP) progression, local inflammatory cell responses to common proinflammatory cytokines, including TNF- $\alpha$ , could further enhance the inflammatory response by upregulating the expression of IKK $\beta$  and subsequent activation of NF- $\kappa$ B signaling (20). These data suggest that etanercept may suppress damaging neuroinflammation during PE by disrupting the TNF- $\alpha$ /NF- $\kappa$ B signaling pathway, but this has not been demonstrated directly. Therefore, the aim of the current study was to assess the effect of etanercept on neuroinflammation and nerve cell protection in a caerulein-induced PE model.

## Materials and methods

**Animals and groups.** The Jinan University Laboratory Animal Welfare and Ethics Committee charter approved all procedures involving rats. All protocols also conformed to the guidelines of Animal Use and Care of the National Institutes of Health (21). *In vivo* experiments were conducted on 8-week-old male Sprague–Dawley (SD) rats (weight, 250–300 g) purchased from the Southern Medical University animal center (Guangzhou, Guangdong). Rats were housed in rectangular polypropylene cages under a controlled temperature (20 $\pm$ 1°C), with a humidity of 50–60% and a 12-h light/dark cycle with *ad libitum* access to food and water. The mortality rate of the animals during the modeling period was 58%; animals were euthanized when they reached a humane endpoint (22). Rats were anesthetized or euthanized with intraperitoneal (IP) injection of pentobarbital sodium (50 or 120 mg/kg, respectively) based on previously described methods (23,24). To confirm the vital signs of experimental animals, the vital signs (including spontaneous breathing and pupil size) were observed on an hourly basis. No spontaneous breathing, heartbeat, dilated pupils, and direct and indirect

light reflection of bilateral pupils were used to indicate that the animals had succumbed. Finally, the surviving experimental animals were euthanized by IP injection of pentobarbital sodium and then the tissue samples were quickly collected. Dead animals and euthanized animals were placed in waste bags, temporarily stored in freezers, and finally recycled and disposed of by the University Experimental Animal Center. In the process of modeling, the main causes of death in experimental animals were considered as brain edema, cerebral hernia and shock. All the surgical interventions were performed under sterile conditions and strictly complied with the requirements of NC3Rs guidelines (25).

A total of 30 SD rats were randomly separated into three groups (n=10/group): The sham group was the control group, and was subjected to neck skin incision and closure, followed by injection with an equivalent volume of saline. The Caerulein group was the PE model group (n=10) and received the same surgical procedure, but were injected with 50  $\mu$ g/kg caerulein (26) through the jugular vein. The Caerulein + EP group was the PE model + EP group and received the same surgical procedure, but was treated with 50  $\mu$ g/kg caerulein and 10 mg/kg etanercept (Immunex Corporation) (27) through the jugular vein. All animals were sacrificed at 6 h after injection according to the previous literature (28) for histochemical and biochemical analyses.

**Cell culture and treatment.** Rat hippocampal H19–7/IGF-IR cells (29) were obtained from the American Type Culture Collection (cat. no. CRL-2526<sup>TM</sup>) and cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 0.2 mg/ml G418, 0.001 mg/ml puromycin (Invitrogen; Thermo Fisher Scientific, Inc.) and 10% fetal calf serum (Invitrogen; Thermo Fisher Scientific, Inc.). The culture medium was replaced every 2 days. H19–7/IGF-IR cells were seeded at 2 $\times$ 10<sup>5</sup>/well on 6-well plates coated with 0.015 mg/ml poly-L-lysine and cultured in a humidified incubator at 34°C under a 5% CO<sub>2</sub> atmosphere for 3–5 days. Cells were then treated with caerulein (10 nmol/ml) alone or in combination with 1, 10 or 100  $\mu$ mol/ml etanercept according to previous studies (30,31). At 6 h after administration, cells were washed with PBS and collected for further investigation.

**Histological analysis.** Pathogenic changes in the hippocampus, and the expression levels of various proinflammatory and neurotrophic factors were examined by hematoxylin and eosin (H&E) staining and immunohistochemical staining, respectively. Briefly, rats were euthanized by IP injection of pentobarbital sodium (120 mg/kg) and perfused transcardially with 100 ml physiological saline. The hippocampus was isolated and immersed in 0.1 M PBS (pH 7.4) with 4% paraformaldehyde and 30% sucrose overnight at 4°C. Tissues were then embedded in optimal cutting temperature compound (Sakura Finetek) and stored at -80°C. Horizontal and coronal sections (5  $\mu$ m) were prepared for immunohistochemical and H&E staining for 5 min at room temperature. Each section was washed with PBS plus 0.1% Triton X-100 for 7 min and then blocked for 1 h in 10% bovine serum albumin (BSA; Sigma-Aldrich; Merck KGaA) at room temperature. Sections were incubated overnight at 4°C with the following

primary antibodies (all from Abcam): Anti-IL-1 $\beta$  (1:1,000; cat. no. ab9722); TNF- $\alpha$  (1:1,000; cat. no. ab6671); IL-6 (1:1,000; cat. no. ab9324); and hypoxia-inducible factor 1  $\alpha$  (HIF-1 $\alpha$ ; 1:1,000; cat. no. ab8366). Following washing in PBS, sections were incubated in secondary antibodies: Anti-mouse IgG H&L [(horseradish peroxidase; HRP) 1:2,000; cat. no. ab205719; Abcam] and anti-Rabbit IgG H&L [(HRP); 1:2,000; cat. no. ab6721; Abcam], with 2% BSA for 1 h at room temperature and then washed again in PBS. Immunolabeling was revealed using DAB as a chromogen for light microscopy. H&E staining was conducted according to the manufacturer's instructions of H&E staining kit (cat. no. C0105; Beyotime Institute of Biotechnology). All sections were viewed under an Olympus BX51 microscope (Olympus Corporation; magnification, x100) and six slices were randomly selected for each group.

**RNA extraction, cDNA synthesis and reverse transcription-quantitative (RT-q)PCR.** Total RNA was extracted from rat hippocampal H19-7/IGF-IR cells using TRIzol<sup>®</sup> reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. A Reverse Transcriptase Reagent kit (Takara Bio, Inc.; cat. no. RR037A) was used to synthesize cDNA according to the manufacturer's instructions. SYBR Green Master mix kits (Takara Bio, Inc.; cat. no. RR820A) were used to assess the efficiency of RT-qPCR. Reactions were conducted in 200  $\mu$ l thin-walled reaction tubes using an ABI Prism 7500 Sequence Detection System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The reaction mixture contained 2X SYBR Green Master mix (10  $\mu$ l), sense and antisense primers (0.8  $\mu$ l, 2.5  $\mu$ M of each) and cDNA (5  $\mu$ l, 10 ng). The thermocycling included initial denaturation at 95°C for 60 sec, followed by 40 cycles of denaturation at 95°C for 5 sec and annealing/extension at 60°C for 30 sec. GAPDH was used as the internal control gene. Relative gene expression was calculated using the  $2^{-\Delta\Delta C_t}$  method (32). The primers used for amplification were: IL-1 $\beta$  forward, 5'-CCAGGATGAGGACCAAGCA-3' and reverse, 5'-TCCCGACCATTGCTGTTCC-3'; TNF- $\alpha$  forward, 5'-AAGCCCGTAGCCACGTCGTA-3' and reverse, 5'-GCCCGCAATCCAGGCCACTAC-3'; IL-6 forward, 5'-GATTTACATAAAAATAGTCCTTCCCTACC-3' and reverse, 5'-GGTTTGCCGAGTAGATCTCAAAGTG-3'; HIF1- $\alpha$  forward, 5'-ACCTATGACCTGCTTGGTGCTGAT-3' and reverse, 5'-CAGTTTCTGTGTCGTTGCTGCCAA-3';  $\beta$ -actin forward, 5'-ACCATTGGCAATGAGCGGT-3' and reverse, 5'-GTCTTTGCGGATGTCCACGT-3'.

**ELISA.** Blood samples (1 ml) were collected and centrifuged at 1,000 x g for 15 min at 4°C to extract serum. The samples were stored at -80°C before the detection. Blood samples (three rat blood samples in each group) were used to detect the expression of amylase (cat. no. E-EL-R2544c; Elabscience), TNF- $\alpha$  (cat. no. ab100785; Abcam) and IL-6 (cat. no. ab100772; Abcam) according to the manufacturer's protocols.

**Western blot assay.** Western blotting was used to detect protein expression and phosphorylation status. H19-7/IGF-IR cells in 6-well plates ( $2 \times 10^5$  cells/well) treated as described were submerged in ice-cold homogenization buffer containing 50 mM Tris-HCl (pH 7.5), 0.5% Triton X-100, and phosphatase/protease inhibitor cocktail (Roche Diagnostics), and centrifuged (10,000 x g, 20 min, 4°C). The supernatants were transferred into new tubes and total protein concentration was determined using bicinchoninic acid protein assay kit (cat. no. 23227; Thermo Fisher Scientific, Inc.). Then, 30  $\mu$ g of protein were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad Laboratories, Inc.). The membranes were blocked using 5% milk (cat. no. MA0097-400ML; Dalian Meilunbio Co., Ltd.) at 37°C for 1 h. Then, incubated with species-specific primary antibodies, including rabbit anti IL-1 $\beta$  (1:1,000; cat. no. ab9722; Abcam), TNF- $\alpha$  (1:1,000; cat. no. ab6671; Abcam), IL-6 (1:1,000; cat. no. ab9324; Abcam), HIF1- $\alpha$  (1:1,000; cat. no. ab179483; Abcam), phosphorylated (p)-NF- $\kappa$ B (1:1,000; cat. no. 3033; Cell Signaling Technology, Inc.), NF- $\kappa$ B (1:1,000; cat. no. 8242; Cell Signaling Technology, Inc.), Anti- $\beta$ -actin (1:1,000; cat. no. ab115777; Abcam), p-IKK $\beta$  (1:1,000; cat. no. 2697; Cell Signaling Technology, Inc.) and IKK $\beta$  (1:1,000; cat. no. 2678; Cell Signaling Technology, Inc.) overnight at 4°C. Membranes were then incubated with species-specific horseradish peroxidase conjugated secondary antibodies goat anti-mouse (1:2,000; Abcam; cat. no. ab6789) and goat anti-rabbit (1:2,000; Abcam; cat. no. ab6721). Protein bands were visualized using an ECL kit (Pierce; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions and exposed to X-ray films.  $\beta$ -actin was used as a protein loading control.

tase/protease inhibitor cocktail (Roche Diagnostics), and centrifuged (10,000 x g, 20 min, 4°C). The supernatants were transferred into new tubes and total protein concentration was determined using bicinchoninic acid protein assay kit (cat. no. 23227; Thermo Fisher Scientific, Inc.). Then, 30  $\mu$ g of protein were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad Laboratories, Inc.). The membranes were blocked using 5% milk (cat. no. MA0097-400ML; Dalian Meilunbio Co., Ltd.) at 37°C for 1 h. Then, incubated with species-specific primary antibodies, including rabbit anti IL-1 $\beta$  (1:1,000; cat. no. ab9722; Abcam), TNF- $\alpha$  (1:1,000; cat. no. ab6671; Abcam), IL-6 (1:1,000; cat. no. ab9324; Abcam), HIF1- $\alpha$  (1:1,000; cat. no. ab179483; Abcam), phosphorylated (p)-NF- $\kappa$ B (1:1,000; cat. no. 3033; Cell Signaling Technology, Inc.), NF- $\kappa$ B (1:1,000; cat. no. 8242; Cell Signaling Technology, Inc.), Anti- $\beta$ -actin (1:1,000; cat. no. ab115777; Abcam), p-IKK $\beta$  (1:1,000; cat. no. 2697; Cell Signaling Technology, Inc.) and IKK $\beta$  (1:1,000; cat. no. 2678; Cell Signaling Technology, Inc.) overnight at 4°C. Membranes were then incubated with species-specific horseradish peroxidase conjugated secondary antibodies goat anti-mouse (1:2,000; Abcam; cat. no. ab6789) and goat anti-rabbit (1:2,000; Abcam; cat. no. ab6721). Protein bands were visualized using an ECL kit (Pierce; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions and exposed to X-ray films.  $\beta$ -actin was used as a protein loading control.

**Detection of reactive oxygen species (ROS) level.** Cellular ROS level was measured fluorometrically using the ROS sensitive dye 2'-7'-dichlorodihydrofluorescein diacetate (DCFH-DA) as described by Chen *et al* (33). Briefly, H19-7/IGF-IR cells treated as indicated were collected, centrifuged at 500 x g for 5 min at room temperature, resuspended in PBS with DCFH-DA (10  $\mu$ M), and incubated at room temperature for 30 min in darkness. ROS levels were measured by flow cytometry using a BD FACSCalibur flow cytometer (BD Biosciences) with excitation set at 488 nm and emission at 530 nm and analyze by Flowjo V10 software (34).

**Statistical analyses.** All data are expressed as mean  $\pm$  standard deviation of at least three independent experiments. Student's t-test and one-way ANOVA followed by Bonferroni's post hoc test were used to compare treatment group means. P<0.05 was considered to indicate a statistically significant difference. All statistical calculations were performed using SPSS 21.0 (IBM Corp.).

## Results

**Effects of etanercept treatment on proinflammatory cytokine release in caerulein-induced PE in vitro.** To investigate the role of etanercept in PE pathogenesis *in vitro*, the present study employed a PE model with caerulein (10 nmol/l) administration using rat hippocampal H19-7/IGF-IR cells treated with different concentrations of etanercept (1, 10 and 100  $\mu$ mol/ml). At 6 h after etanercept administration in caerulein-induced H19-7/IGF-IR cells, the proinflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$  and IL-6 were examined by immunoblotting and RT-qPCR analysis. The effects of etanercept

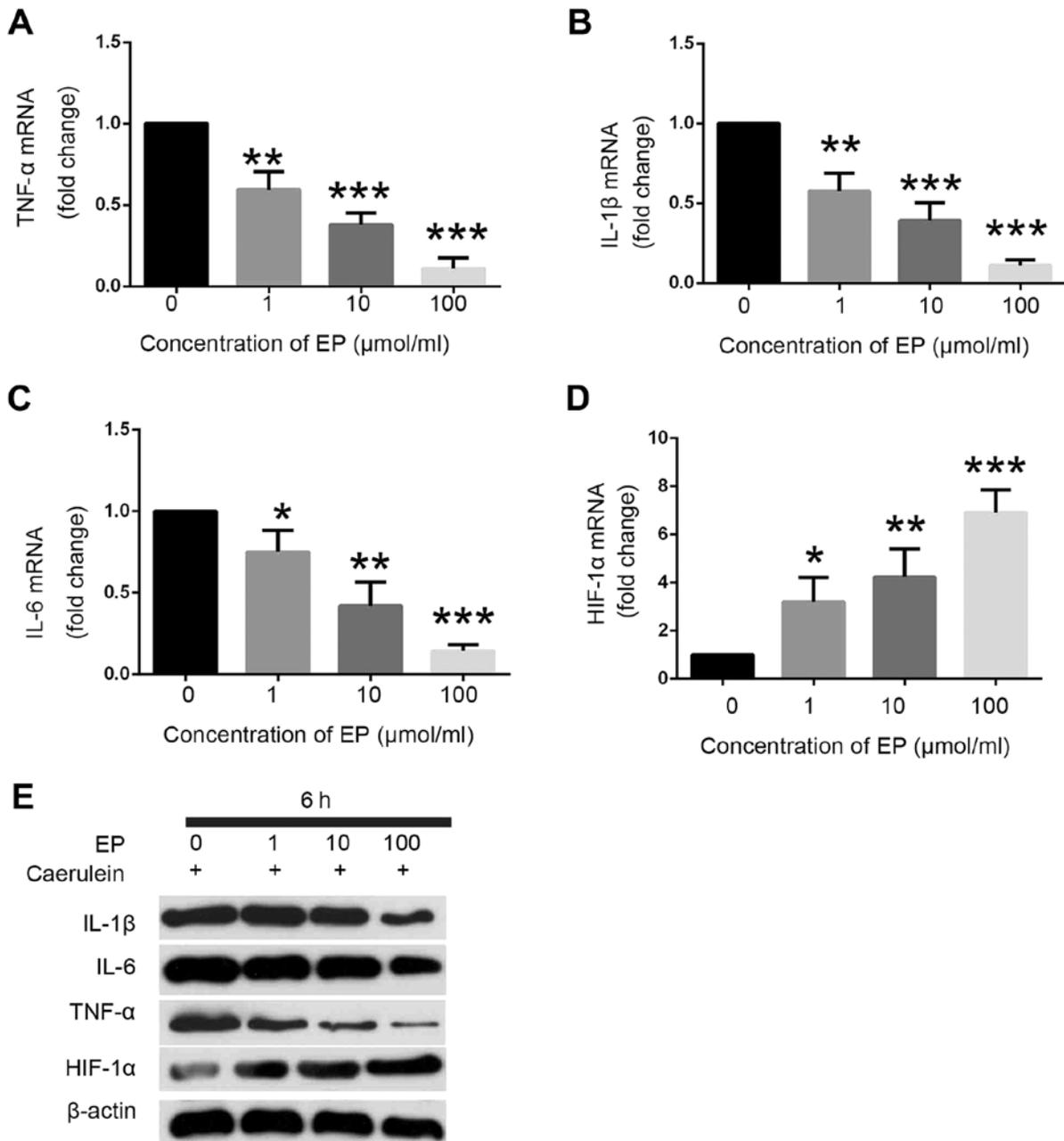


Figure 1. Etanercept attenuates caerulein-induced decreases in proinflammatory cytokine release. Etanercept (1, 10, or 100 μmol/ml) dose-dependently downregulated mRNA expression levels of (A) TNF-α, (B) IL-1β and (C) IL-6 in a caerulein-induced (10 nmol/l) model of pancreatic encephalopathy in H19-7/IGF-IR cells, as measured by RT-qPCR at 6 h after etanercept treatment. (D) Etanercept (1, 10, or 100 μmol/ml) dose-dependently enhanced HIF-1α mRNA expression in caerulein-induced (10 nmol/l) H19-7/IGF-IR cells, as measured by RT-qPCR at 6 h after etanercept treatment. N=3; \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 vs. group without Etanercept. (E) Etanercept (1, 10, or 100 μmol/ml) dose-dependently downregulated protein expression levels of IL-1β, IL-6, and TNF-α, and upregulated HIF-1α protein expression, as determined via western blotting in caerulein-induced (10 nmol/l) H19-7/IGF-IR cells at 6 h after etanercept treatment. Data are presented as mean ± standard deviation. Experiments were repeated three times for reproducibility. TNF, tumor necrosis factor; RT-qPCR, reverse transcription-quantitative PCR; IL, interleukin; HIF-1α, hypoxia-inducible factor 1 α; EP, etanercept.

on the expression of the neuroprotective factor HIF-1α were also examined. It was determined that the mRNA levels of TNF-α, IL-1β and IL-6 were significantly reduced in caerulein-induced H19-7/IGF-IR cells following etanercept treatment (Fig. 1A-C). By contrast, HIF-1α mRNA expression levels were significantly increased in caerulein-induced H19-7/IGF-IR cells following etanercept treatment (Fig. 1D). It was found that etanercept effectively altered the mRNA levels of TNF-α, IL-1β, IL-6 and HIF-α in a dose-dependent manner in caerulein-induced H19-7/IGF-IR cells. The

protein levels of proinflammatory cytokines were confirmed by western blot analysis. It was identified that the protein expression levels of TNF-α, IL-1β and IL-6 were attenuated, whereas those of HIF-1α were enhanced (Fig. 1E). The effects of etanercept administration on protein expression levels were dose-dependent. Taken together, these data indicated that etanercept can contribute to the activation of HIF-1α and inhibit the activation of proinflammatory factors, including TNF-α, IL-1β and IL-6; thus, it may work as an anti-inflammatory factor in clinic disease treatment.

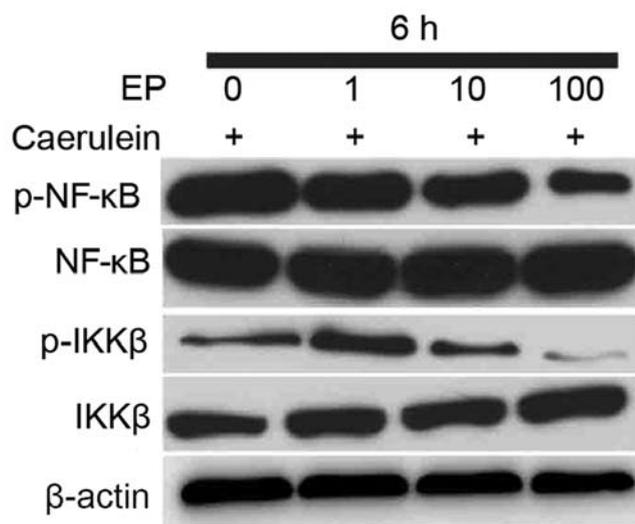


Figure 2. Etanercept attenuates caerulein-induced inhibition of the NF- $\kappa$ B signaling pathway. Western blots showing downregulated p-NF- $\kappa$ B and p-IKK $\beta$  protein expression in a caerulein-induced model of pancreatic encephalopathy in H19-7/IGF-IR cells, which was negatively regulated by etanercept in a dose-dependent manner (n=3). p-, phosphorylated; IKK $\beta$ , I $\kappa$ B kinase.

*Effects of etanercept treatment on ROS in caerulein-induced PE in vitro.* Oxidative stress, and ensuing accumulation of DNA lesions and damage to other macromolecules may contribute to PE pathogenesis (35). To investigate whether etanercept can suppress caerulein-induced oxidative stress in H19-7/IGF-IR cells, DCFH-DA fluorescence was compared among cultures treated with 10 nmol/ml caerulein alone or combined with 1, 10, or 100  $\mu$ mol/ml etanercept by flow cytometry (Fig. S1A-D). Flow cytometry showed the peak shifted to the left with the increased concentration of etanercept. Indeed, etanercept dose-dependently reduced caerulein-induced ROS accumulation in H19-7/IGF-IR cells (Fig. S1E).

*Effects of etanercept treatment on the NF- $\kappa$ B signaling pathway in caerulein-induced PE in vitro.* NF- $\kappa$ B is a master regulator of inflammatory cytokine expression, and contributes to the progression of neuroinflammation and ensuing neuronal damage (36). Therefore, it was examined whether etanercept altered caerulein-induced phosphorylation (activation) of NF- $\kappa$ B and the upstream effector IKK $\beta$ . At 6 h after treatment with etanercept, western blot analysis indicated downregulated expression of p-IKK $\beta$  and p-NF- $\kappa$ B in caerulein-induced H19-7/IGF-IR cells (Fig. 2). As expected, the phosphorylation of p-IKK $\beta$  and p-NF- $\kappa$ B reduced in line with the increased concentration of etanercept. These findings identified the dominant role of etanercept in negatively regulating the NF- $\kappa$ B signaling pathway in caerulein-induced H19-7/IGF-IR cells *in vitro*.

*Effects of etanercept treatment on hippocampus cell inflammatory factor expression in caerulein-induced PE in vivo.* Rats were injected with caerulein (50  $\mu$ g/kg) alone or combined with etanercept (10 mg/kg). At 6 h after injection, hippocampal inflammation and damage were detected via ELISA and histochemical analysis. ELISAs revealed decreased expression levels of amylase, TNF- $\alpha$  and IL-6 in

the Caerulein + EP group compared with the Caerulein group (Fig. 3A-C). Furthermore, immunohistochemical analysis demonstrated elevated expression levels of IL-1 $\beta$  (Fig. 3D), TNF- $\alpha$  (Fig. 3E) and IL-6 (Fig. 3F) in hippocampal neurons in the Caerulein group, which were reversed by cotreatment with etanercept in the Caerulein + EP group. However, etanercept enhanced expression of HIF-1 $\alpha$  in hippocampus cells in the Caerulein + EP group compared with the Caerulein group (Fig. 3G). Therefore, etanercept can inhibit caerulein-induced PE progression and decrease the release of inflammatory factors in hippocampus cells.

## Discussion

Inflammatory and autoimmune processes are critical to the pathogenesis and progression of AP, but it is not fully understood how the progression of AP leads to PE. PE is among the most serious complications of AP (37), characterized by neurodegeneration and psychiatric manifestations including orientation disorders, trance, excitement and hallucinations (38). The mortality rate of AP with mental and neurological symptoms can reach 67-100%, as reported in China (39). Thus, models allowing for elucidation of PE pathogenesis are essential for improving treatment strategies. The current study demonstrated that etanercept had an important role in caerulein-triggered immune reactions and TNF- $\alpha$  signaling for PE-associated neural damage and that it provides a potential therapeutic treatment for anti-inflammatory disease.

Previous studies have shown that proinflammatory cytokines can cause neurovegetative states and depressive symptoms (40,41). The elevated activity of proinflammatory cytokines and associated signaling pathways in inflammatory disorders may aggravate depression and decrease the response to conventional antidepressant medications (42). In AP, acinar cell injury initiates local inflammatory and immune responses, and accelerates the release of proinflammatory cytokines, including TNF- $\alpha$  and IL-6 (43). The activated cytokine cascades can accelerate systemic inflammatory responses and multisystem failure independently from AP development (44). Though these processes, patients with AP may experience changes in consciousness and eventual PE (45).

Clinical studies have reported associations between serum TNF- $\alpha$  and the severity of disease such as multiple sclerosis (46), acquired immunodeficiency syndrome (AIDS) (47) and malaria (48), as well as associations between serum proinflammatory cytokine concentrations and central nervous system damage. For instance, patients with systemic lupus erythematosus exhibiting neural sequela (49) and patients with AIDS with associated encephalopathy exhibit markedly elevated serum and cerebrospinal fluid IL-6 levels (50). In addition, TNF- $\alpha$  and IL-6 expression levels are notably increased in patients with severe head trauma (45). Local oxidative stress appears to be a major final common pathway for neuronal damage in these disorders. Using flow cytometry analysis, the current study demonstrated that etanercept could prevent the accumulation of ROS in caerulein-induced PE.

TNF- $\alpha$  is hypothesized to be directly or indirectly responsible for the majority of inflammatory and destructive events in rheumatoid arthritis (RA) (51). For instance, TNF- $\alpha$  controls the expression of IKK $\beta$  in esophageal epithelia, regulates the

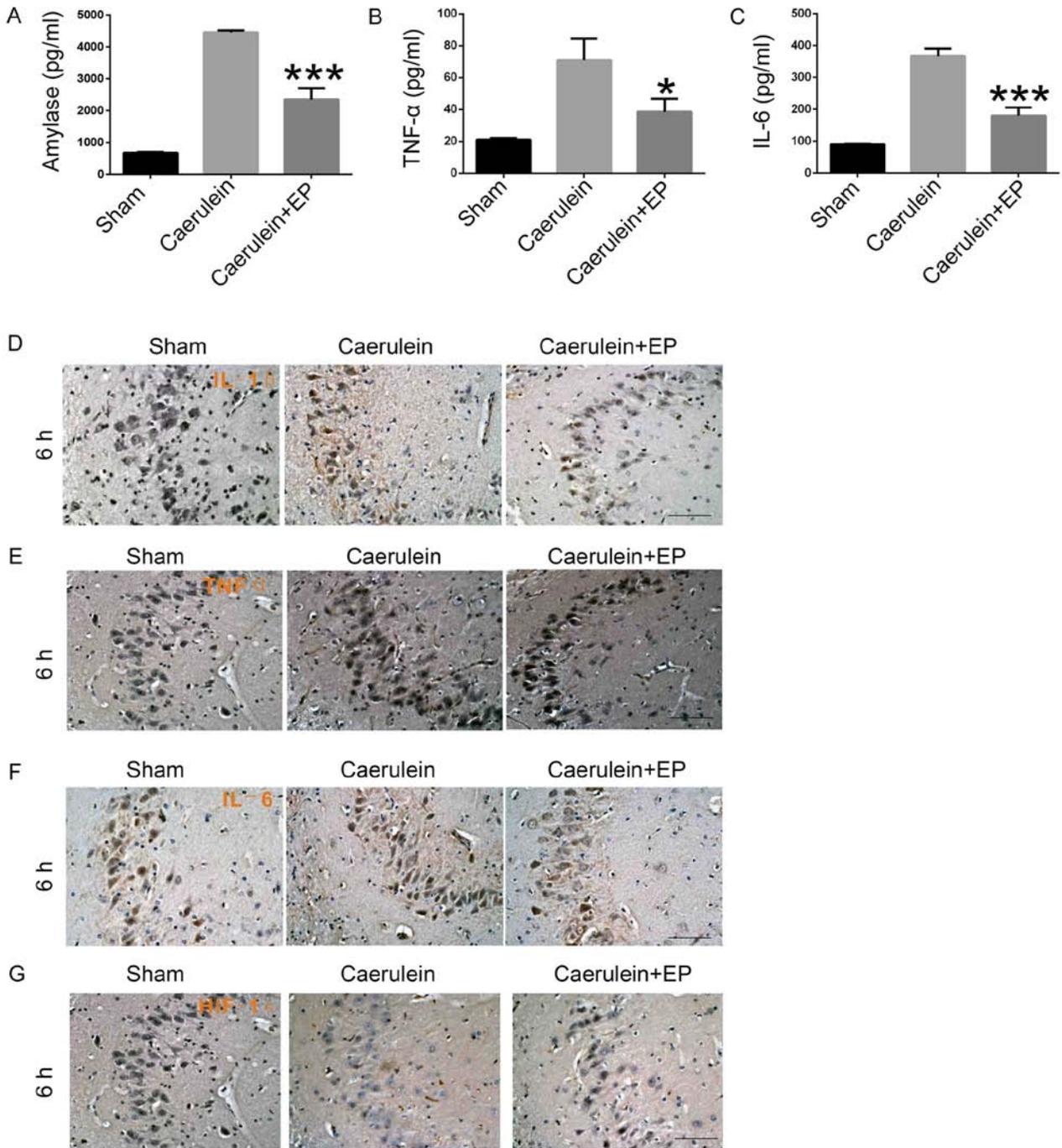


Figure 3. Etanercept attenuates caerulein-induced inflammatory factor expression in hippocampus cells in a rat model of pancreatic encephalopathy. ELISA analysis revealed an increase in the expression of (A) amylase, (B) TNF- $\alpha$  and (C) IL-6 following 50  $\mu$ g/kg caerulein injection (Caerulein group). These signs of neural damage were reversed by co-treatment with 10 mg/kg etanercept (Caerulein + EP group). N=3; \*P<0.05, \*\*\*P<0.001 vs. Caerulein group. Immunohistochemical staining revealed the upregulated expression of (D) IL-1 $\beta$ , (E) TNF- $\alpha$  and (F) IL-6 in the Caerulein group compared with the Sham group, which was reversed by co-treatment with 10 mg/kg EP. (G) Immunohistochemical staining revealed the upregulated expression of HIF-1 $\alpha$  in hippocampal cells in the Caerulein + EP group, compared with the Caerulein group. Scale bars, 100  $\mu$ m (n=3). TNF, tumor necrosis factor; EP, etanercept; IL, interleukin.

activation of human endothelial cells (52,53) and stimulates neoangiogenesis (54,55), which leads to an influx of inflammatory cells into the rheumatoid joint. On the other hand, ensuing local immune reactions increase the production of enzymes responsible for the degradation of cartilage, such as matrix metalloproteinases (56), and activate osteoclasts, thereby enhancing bone resorption. In addition, TNF- $\alpha$  induces the production of proinflammatory cytokines, including IL-1 and

IL-6 (57,58), and increases prostaglandin E2 production (59). TNF- $\alpha$  and IL-6 serve as important early markers for AP and the severity of its complications (60,61). Patients with severe depression also exhibit enhanced levels of proinflammatory cytokines in peripheral blood (62). These cytokines can enter the brain and contribute to the development of depression by affecting indolamine 2,3-dioxygenase and neurotransmitter metabolism (63).

The TNF- $\alpha$  inhibitor etanercept is approved clinically for the treatment of plaque psoriasis, RA, psoriatic arthritis and axial spondyloarthritis (64). Etanercept can alleviate arthritic signs and symptoms, thus reducing disease-associated disability and promoting health-associated quality of life. Further, these benefits appear to be maintained during long-term treatment (43). Similarly, arthritic symptoms were significantly improved by etanercept compared to a placebo, according to subjective and objective assessments of disease activity and physical function (65). These benefits may be further augmented by combination therapy using methotrexate. The efficacy of etanercept against RA is also rapid and can be observed as early as 1 week after initiating therapy (66). Its interaction with the immune system by associating with anxiety and the activation of auditory evoked potentials, which may increase the levels of proinflammatory cytokines such as TNF- $\alpha$  and IL-6 (67). Injection of IL-1 $\alpha$  could block the function of IL-1 $\beta$  and significantly reduce the expression of NF- $\kappa$ B in the medial prefrontal cortex, thus significantly improving depressive behaviors in rats (68). Previous studies have shown that etanercept can be used in the treatment of serum-positive RA and Stevens-Johnson syndrome/toxic epidermolysis triggered by carbamazepine, and play a key role in cardiovascular safety in patients with RA (69-71). However, there are few cases of clinical treatment of PE with etanercept.

In the current study, etanercept was used to evaluate the role of TNF- $\alpha$ -mediated inflammation in caerulein-induced PE. The results demonstrated that etanercept can act as an anti-inflammatory and protective agent against PE-associated neural dysfunction in the suppression of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 expression, *in vitro* and *in vivo*. In addition, inhibition of TNF- $\alpha$  led to the attenuation of NF- $\kappa$ B signaling and amelioration of oxidative stress. However, etanercept promoted the induction of HIF-1 $\alpha$ , which is released by mononuclear cells and/or neutrophils, and consequently reduces leukocyte migration at the site of the inflammatory response (72). In order to confirm the hypothesis of the present study, it is necessary to consider more literature on the scoring indicators of PE, such as the blood-brain barrier score (73); this will be performed in future studies. Taken together, etanercept effectively improves caerulein-induced PE by suppressing inflammatory responses, and could act as a safe and effective therapeutic option in the development of therapeutic strategies for the treatment of PE-related disease.

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#### Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

#### Authors' contributions

XW designed the study. YL, HL, GZ and YX were involved in data acquisition and conducted the experiments. GJ, BL and CL analyzed the data. YL drafted the article. XW was involved in the final approval of the submitted manuscript. All authors read and approved the final manuscript.

#### Ethics approval and consent to participate

The Jinan University Laboratory Animal Welfare and Ethics Committee charter approved all procedures involving rats. All protocols also conformed to the guidelines of Animal Use and Care of the National Institutes of Health.

#### Patient consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

#### References

- Colmant HJ and Noltenius H: Pancreatic encephalopathy. *Med Klin* 72: 2146-2154, 1977 (In German).
- Zhang XP and Tian H: Pathogenesis of pancreatic encephalopathy in severe acute pancreatitis. *Hepatobiliary Pancreat Dis Int* 6: 134-140, 2007.
- Mentula P and Leppäniemi A: Position paper: Timely interventions in severe acute pancreatitis are crucial for survival. *World J Emerg Surg* 9: 15, 2014.
- Zheng L, Xue J, Jaffee EM and Habtezion A: Role of immune cells and immune-based therapies in pancreatitis and pancreatic ductal adenocarcinoma. *Gastroenterology* 144: 1230-1240, 2013.
- Al Mofleh IA: Severe acute pancreatitis: Pathogenetic aspects and prognostic factors. *World journal of gastroenterology* 14: 675-684, 2008.
- Zhang H, Neuhöfer P, Song L, Rabe B, Lesina M, Kurkowski MU, Treiber M, Wartmann T, Regnér S, Thorlacijs H, *et al*: IL-6 trans-signaling promotes pancreatitis-associated lung injury and lethality. *J Clin Invest* 123: 1019-1031, 2013.
- Wang X, Zhuang X, Wei R, Wang C, Xue X and Mao L: Protective effects of *Acanthopanax* vs. Ulinastatin against severe acute pancreatitis-induced brain injury in rats. *Int Immunopharmacol* 24: 285-298, 2015.
- Ohkubo T, Shiojiri T and Matsunaga T: Severe diffuse white matter lesions in a patient with pancreatic encephalopathy. *J Neurol* 251: 476-478, 2004.
- Adler G, Hahn C, Kern HF and Rao KN: Cerulein-induced pancreatitis in rats: Increased lysosomal enzyme activity and autophagocytosis. *Digestion* 32: 10-18, 1985.
- Yin T, Peeters R, Liu Y, Feng Y, Zhang X, Jiang Y, Yu J, Dymarkowski S, Himmelreich U, Oyen R and Ni Y: Visualization, quantification and characterization of caerulein-induced acute pancreatitis in rats by 3.0T clinical MRI, biochemistry and histomorphology. *Theranostics* 7: 285-294, 2017.
- Marrache F, Tu SP, Bhagat G, Pendyala S, Osterreicher CH, Gordon S, Ramanathan V, Penz-Osterreicher M, Betz KS, Song Z and Wang TC: Overexpression of interleukin-1 $\beta$  in the murine pancreas results in chronic pancreatitis. *Gastroenterology* 135: 1277-1287, 2008.
- Terao K, Wake H, Adachi N, Liu K, Teshigawara K, Takahashi H, Mori S and Nishibori M: Histidine-rich glycoprotein suppresses hyperinflammatory responses of lung in a severe acute pancreatitis mouse model. *Pancreas* 47: 1156-1164, 2018.
- Hoesel B and Schmid JA: The complexity of NF- $\kappa$ B signaling in inflammation and cancer. *Mol Cancer* 12: 86, 2013.
- Cildir G, Low KC and Tergaonkar V: Noncanonical NF- $\kappa$ B signaling in health and disease. *Trends Mol Med* 22: 414-429, 2016.

15. Hayden MS and Ghosh S: NF- $\kappa$ B in immunobiology. *Cell Res* 21: 223-244, 2011.
16. Cui X, Chang L, Li Y, Lv Q, Wang F, Lin Y, Li W, Meade JD, Walden JC and Liang P: Trivalent soluble TNF receptor, a potent TNF-alpha antagonist for the treatment collagen-induced arthritis. *Sci Rep* 8: 7327, 2018.
17. Zhao S, Mysler E and Moots RJ: Etanercept for the treatment of rheumatoid arthritis. *Immunotherapy* 10: 433-445, 2018.
18. Kovar FM, Marsik C, Cvitko T, Wagner OF, Jilma B and Endler G: The tumor necrosis factor alpha-308 G/A polymorphism does not influence inflammation and coagulation response in human endotoxemia. *Shock* 27: 238-241, 2007.
19. Sheng YQ, Li F and Qin ZH: TNF receptor 2 makes tumor necrosis factor a friend of tumors. *Front Immunol* 9: 1170, 2018.
20. Muniraj T, Gajendran M, Thiruvengadam S, Raghuram K, Rao S and Devaraj P: Acute pancreatitis. *Dis Mon* 58: 98-144, 2012.
21. National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals. The National Academies Collection: Reports funded by National Institutes of Health. In: Th, editor. *Guide for the Care and Use of Laboratory Animals*. Washington (DC): National Academies Press (US) National Academy of Sciences, 2011.
22. How to determine humane endpoints for research animals. *Lab Anim (NY)* 45: 19, 2016.
23. Sano Y, Ito S, Yoneda M, Nagasawa K, Matsuura N, Yamada Y, Uchinaka A, Bando YK, Murohara T and Nagata K: Effects of various types of anesthesia on hemodynamics, cardiac function, and glucose and lipid metabolism in rats. *Am J Physiol Heart Circ Physiol* 311: H1360-H1366, 2016.
24. Pierozan P, Jerneerén F, Ransome Y and Karlsson O: The choice of euthanasia method affects metabolic serum biomarkers. *Basic Clin Pharmacol Toxicol* 121: 113-118, 2017.
25. NC3Rs Reporting Guidelines Working Group: Animal research: Reporting in vivo experiments: The ARRIVE guidelines. *J Physiol* 588: 2519-2521, 2010.
26. Orabi AI, Shah AU, Ahmad MU, Choo-Wing R, Parness J, Jain D, Bhandari V and Husain SZ: Dantrolene mitigates caerulein-induced pancreatitis in vivo in mice. *Am J Physiol Gastrointest Liver Physiol* 299: G196-G204, 2010.
27. Jeong YK, Lee S, Lim JW and Kim H: Docosahexaenoic acid inhibits cerulein-induced acute pancreatitis in rats. *Nutrients* 9: E744, 2017.
28. Huang HL, Tang GD, Liang ZH, Qin MB, Wang XM, Chang RJ and Qin HP: Role of Wnt/ $\beta$ -catenin pathway agonist SKL2001 in Caerulein-induced acute pancreatitis. *Can J Physiol Pharmacol* 97: 15-22, 2019.
29. Morrione A, Navarro M, Romano G, Dews M, Reiss K, Valentini B, Belletti B and Baserga R: The role of the insulin receptor substrate-1 in the differentiation of rat hippocampal neuronal cells. *Oncogene* 20: 4842-4852, 2001.
30. DiMaggio MJ, Williams JA, Hao Y, Ernst SA and Owyang C: Endothelial nitric oxide synthase is protective in the initiation of caerulein-induced acute pancreatitis in mice. *Am J Physiol Gastrointest Liver Physiol* 287: G80-G87, 2004.
31. Ye J, Jiang R, Cui M, Zhu B, Sun L, Wang Y, Zohaib A, Dong Q, Ruan X, Song Y, *et al*: Etanercept reduces neuroinflammation and lethality in mouse model of Japanese encephalitis. *J Infect Dis* 210: 875-889, 2014.
32. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>-Delta Delta C(T)</sup> method. *Methods* 25: 402-408, 2001.
33. Chen X, Duan Y, Riley AM, Welch MA, White FA, Grant MB and Obukhov AG: Long-term diabetic microenvironment augments the decay rate of capsaicin-induced currents in mouse dorsal root ganglion neurons. *Molecules* 24: 775, 2019.
34. Fernandez R and Maecker H: Cytokine-stimulated Phosphoflow of PBMC using CyTOF mass cytometry. *Bio Protoc* 5: e1496, 2015.
35. Shi Q, Liao KS, Zhao KL, Wang WX, Zuo T, Deng WH, Chen C, Yu J, Guo WY, He XB, *et al*: Hydrogen-rich saline attenuates acute renal injury in sodium taurocholate-induced severe acute pancreatitis by inhibiting ROS and NF- $\kappa$ B pathway. 2015: 685043, 2015.
36. Karin M and Ben-Neriah Y: Phosphorylation meets ubiquitination: The control of NF- $\kappa$ B activity. *Ann Rev Immunol* 18: 621-663, 2000.
37. Pitchumoni CS, Agarwal N and Jain NK: systemic complications of acute-pancreatitis. *Am J Gastroenterol* 83: 597-606, 1988.
38. Sharma V, Sharma R, Rana SS and Bhasin DK: Pancreatic encephalopathy: An unusual cause of asterixis. *JOP* 15: 383-384, 2014.
39. Zhuan L and Zhaoshen L: Progress in Pancreatic Encephalopathy. *Pancreatolgy* 4: 248-250, 2003.
40. Parlar A, Arslan SO, Doğan MF, Çam SA, Yalçın A, Elibol E, Özer MK, Uçkardeş F and Kara H: The exogenous administration of CB2 specific agonist, GW405833, inhibits inflammation by reducing cytokine production and oxidative stress. *Exp Ther Med* 16: 4900-4908, 2018.
41. Dantzer R, O'Connor JC, Freund GG, Johnson RW and Kelley KW: From inflammation to sickness and depression: When the immune system subjugates the brain. *Nat Rev Neurosci* 9: 46-57, 2008.
42. Song C and Wang H: Cytokines mediated inflammation and decreased neurogenesis in animal models of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 35: 760-768, 2011.
43. Habtezion A: Inflammation in acute and chronic pancreatitis. *Curr Opin Gastroenterol* 31: 395-399, 2015.
44. Liu Y, Li Y, Chen KL, Zhou B, Lv ZY, Zhou ZG and Li Y: Knockdown of Myeloid differentiation factor 88 attenuates lipopolysaccharide-induced inflammatory response in pancreatic ductal cells. *Pancreas* 45: 755-760, 2016.
45. Yan EB, Hellewell SC, Bellander BM, Agyapomaa DA and Morganti-Kossmann MC: Post-traumatic hypoxia exacerbates neurological deficit, neuroinflammation and cerebral metabolism in rats with diffuse traumatic brain injury. *J Neuroinflamm* 8: 147, 2011.
46. Farrokhi M, Etemadifar M, Jafary Alavi MS, Zarkesh-Esfahani SH, Behjati M, Rezaei A and Amani-Beni A: TNF-alpha production by peripheral blood monocytes in multiple sclerosis patients and healthy controls. *Immunol Invest* 44: 590-601, 2015.
47. Gallitano SM, McDermott L, Brar K and Lowenstein E: Use of tumor necrosis factor (TNF) inhibitors in patients with HIV/AIDS. *J Am Acad Dermatol* 74: 974-980, 2016.
48. Kinra P and Dutta V: Serum TNF alpha levels: A prognostic marker for assessment of severity of malaria. *Trop Biomed* 30: 645-653, 2013.
49. Yoshio T, Okamoto H, Kurasawa K, Dei Y, Hirohata S and Minota S: IL-6, IL-8, IP-10, MCP-1 and G-CSF are significantly increased in cerebrospinal fluid but not in sera of patients with central neuropsychiatric lupus erythematosus. *Lupus* 25: 997-1003, 2016.
50. Witwer KW, Sarbanes SL, Liu J and Clements JE: A plasma microRNA signature of acute lentiviral infection: Biomarkers of central nervous system disease. *AIDS* 25: 2057-2067, 2011.
51. Paleolog EM, Young S, Stark AC, McCloskey RV, Feldmann M and Maini RN: Modulation of angiogenic vascular endothelial growth factor by tumor necrosis factor alpha and interleukin-1 in rheumatoid arthritis. *Arthritis Rheum* 41: 1258-1265, 1998.
52. Tétrault MP, Weinblatt D, Ciolino JD, Klein-Szanto AJ, Sackey BK, Twyman-Saint Victor C, Karakasheva T, Teal V and Katz JP: Esophageal expression of active I $\kappa$ B Kinase- $\beta$  in mice up-regulates tumor necrosis factor and granulocyte-macrophage colony-stimulating factor, promoting inflammation and angiogenesis. *Gastroenterology* 150: 1609-1619.e11, 2016.
53. Pober JS, Gimbrone MA Jr, Lapierre LA, Mendrick DL, Fiers W, Rothlein R and Springer TA: Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. *J Immunol* 137: 1893-1896, 1986.
54. Lupia E, Montrucchio G, Battaglia E, Modena V and Camussi G: Role of tumor necrosis factor-alpha and platelet-activating factor in neoangiogenesis induced by synovial fluids of patients with rheumatoid arthritis. *Eur J Immunol* 26: 1690-1694, 1996.
55. Strieter RM, Kunkel SL, Showell HJ, Remick DG, Phan SH, Ward PA and Marks RM: Endothelial cell gene expression of a neutrophil chemotactic factor by TNF-alpha, LPS, and IL-1 beta. *Science* 243: 1467-1469, 1989.
56. Sui Y, Lee JH, DiMicco MA, Vanderploeg EJ, Blake SM, Hung HH, Plaas AH, James IE, Song XY, Lark MW and Grodzinsky AJ: Mechanical injury potentiates proteoglycan catabolism induced by interleukin-6 with soluble interleukin-6 receptor and tumor necrosis factor alpha in immature bovine and adult human articular cartilage. *Arthritis Rheum* 60: 2985-2996, 2009.
57. Yuan JT, Wang L, Lin YJ, Chen JH and Hu JH: Differences of plasma IL-1 and TNF- $\alpha$  in healthy Chinese population. *Open Med (Wars)* 10: 306-310, 2015.

58. Ishitsuka Y, Kawachi Y, Maruyama H, Taguchi S, Fujisawa Y, Furuta J, Nakamura Y, Ishii Y and Otsuka F: Pituitary tumor transforming gene 1 induces tumor necrosis factor- $\alpha$  production from keratinocytes: Implication for involvement in the pathophysiology of psoriasis. *J Invest Dermatol* 133: 2566-2575, 2013.
59. Dayer JM, Beutler B and Cerami A: Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. *J Exp Med* 162: 2163-2168, 1985.
60. Fusic E, Poropat G, Bilic-Zulle L, Licul V, Milic S and Stimac D: The role of IL-6, 8, and 10, sTNF $\alpha$ , CRP, and pancreatic elastase in the prediction of systemic complications in patients with acute pancreatitis. *Gastroenterol Res Pract* 2013: 282645, 2013.
61. Xue D, Zhang W, Zhang Y, Wang H, Zheng B and Shi X: Adjusting effects of baicalin for nuclear Factor-kappa B and tumor necrosis factor-alpha on rats with caerulein-induced acute pancreatitis. *Mediat Inflamm* 2006: 26295, 2006.
62. Otsuka Y, Kamata K, Minaga K, Takenaka M, Watanabe T and Kudo M: Acute pancreatitis with disturbed consciousness caused by hyperparathyroidism. *Intern Med* 57: 3075-3078, 2018.
63. Miller AH, Maletic V and Raison CL: Inflammation and its discontents: The role of cytokines in the pathophysiology of major depression. *Biol Psychiat* 65: 732-741, 2009.
64. Scott LJ: Etanercept: A review of its use in autoimmune inflammatory diseases. *Drugs* 74: 1379-1410, 2014.
65. Hobbs K, Deodhar A, Wang B, Bitman B, Nussbaum J, Chung J and Collier DH: Randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of etanercept in patients with moderately active rheumatoid arthritis despite DMARD therapy. *Springerplus* 4: 113, 2015.
66. Mease PJ, Gladman DD, Collier DH, Ritchlin CT, Helliwell PS, Liu L, Kricorian G and Chung JB: Etanercept and methotrexate as monotherapy or in combination for psoriatic arthritis: Primary results from a randomized, controlled phase III trial. *Arthritis Rheumatol* 71: 1112-1124, 2019.
67. Soyalp M, Yalcin M, Oter V and Ozgonul A: Investigation of procalcitonin, IL-6, oxidative stress index (OSI) plasma and tissue levels in experimental mild and severe pancreatitis in rats. *Bratisl Lek Listy* 118: 137-141, 2017.
68. Horowitz MA, Wertz J, Zhu D, Cattaneo A, Musaelyan K, Nikkheslat N, Thuret S, Pariente CM and Zunszain PA: Antidepressant compounds can be both pro- and anti-inflammatory in human hippocampal cells. *Int J Neuropsychopharmacol* 18: pyu076, 2014.
69. Giles JT, Sattar N, Gabriel S, Ridker PM, Gay S, Warne C, Musselman D, Brockwell L, Shittu E, Klearman M and Fleming TR: cardiovascular safety of tocilizumab versus etanercept in rheumatoid arthritis: A randomized controlled trial. *Arthritis Rheumatol* 72: 31-40, 2020.
70. Smith KM, Amin S, Jones LK Jr, Lachance DH, Flanagan EP, Jentoft ME and Kantarci OH: Glial fibrillary acidic protein (GFAP) autoimmunity in the setting of seropositive rheumatoid arthritis treated with etanercept. *Neurologist* 24: 152-154, 2019.
71. Pham CH, Gillenwater TJ, Nagengast E, McCullough MC, Peng DH and Garner WL: Combination therapy: Etanercept and intravenous immunoglobulin for the acute treatment of Stevens-Johnson syndrome/toxic epidermal necrolysis. *Burns* 45: 1634-1638, 2019.
72. Yang J, He F, Meng Q, Sun Y, Wang W and Wang C: Inhibiting HIF-1 $\alpha$  decreases expression of TNF- $\alpha$  and caspase-3 in specific brain regions exposed kainic acid-induced status epilepticus. *Cell Physiol Biochem* 38: 75-82, 2016.
73. Genet GF, Bentzer P, Hansen MB, Ostrowski SR and Johansson PI: Effects of propranolol and clonidine on brain edema, blood-brain barrier permeability, and endothelial glycocalyx disruption after fluid percussion brain injury in the rat. *J Trauma Acute Care Surg* 84: 89-96, 2018.