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ORIGINAL ARTICLE

Anti-TNF-α antibody alleviates insulin resistance in rats with sepsis-induced stress hyperglycemia

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Abstract
Purpose To explore the effects and mechanisms of anti-tumor necrosis factor-α (TNF-α) antibody on insulin resistance (IR) in rats with sepsis-induced stress hyperglycemia.
Methods The sepsis-induced stress hyperglycemic rat model was constructed by cecal ligation and puncture combined with the intraperitoneal injection of lipopolysaccharide. The rats were randomly divided into six groups: normal control (NC) group, surgical rats (Cntl) group, high-dose anti-TNF-α antibody therapy (TNF, 6 mg/kg) group, low-dose anti-TNF-α antibody therapy (Tnf, 3 mg/kg) group, insulin therapy (INS) group, and INS + Tnf group. The blood glucose and serum insulin concentrations were detected, followed by analysis of intraperitoneal glucose tolerance test (IPGTT) and hyperinsulinemic–euglycemic clamp. Finally, the expression levels of phospho-Akt (p-Akt), Akt, p-mTOR, mTOR, nuclear factor-κB (NFκB), I kappa beta kinase (IKKβ), and suppressor of cytokine signaling (SOCS-3) were detected by western blotting.
Results There was no significant difference in blood glucose concentrations among these groups, while the serum insulin concentration in TNF and Tnf groups was lower than that in the Cntl group at postoperative 6 h ($P < 0.05$). IPGTT analysis revealed that blood glucose level was lower in the TNF group than that in the Cntl group ($P < 0.05$). The glucose infusion rate in the Cntl group was lower than that in the Tnf and TNF groups ($P < 0.05$). The p-Akt/Akt, p-mTOR/mTOR ratio, and expression levels of NFκB, IKKβ and SOCS-3 were lower in the drug intervention than that in the Cntl group ($P < 0.05$).
Conclusions Anti-TNF-α antibody could reduce IR by inhibiting AKt/mTOR signaling pathway and the expression levels of NFκB, IKKβ, and SOCS-3 in rats with sepsis-induced stress hyperglycemia.

Keywords Stress hyperglycemia · Insulin sensitivity · Anti-TNF-α antibody

Introduction

Stress hyperglycemia is associated with the elevation of blood glucose levels but not preexisting diabetes, and arises from various stresses such as trauma, surgery, burn injury, and sepsis. It is very common in patients with severe sepsis [1] and has deleterious effects on these patients [2, 3]. Acute insulin resistance (IR) is considered as one of the main causes of hyperglycemia [4]. A recent study has reported that intensive insulin therapy at normalization of blood glucose (4.4–6.1 mmol/L) is effective in reducing morbidity and mortality in critically ill patients [5]. However, other study has been reported that the use of intensive insulin therapy in patients with sepsis causes adverse events associated with hypoglycemia and its toxicity increases when the doses accumulated [6]. Therefore, an understanding of the molecular mechanisms of hyperglycemia and acute IR may provide new therapeutic approaches in treating the critically ill following injury.

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Hyperglycemia is known to be implicated in many inflammatory pathways, such as nuclear factor-κB (NF-κB), Akt, mammalian target of rapamycin (mTOR), and I kappa beta kinase (IKKβ). Glucose intake can result in an increase of NFκB and a decrease in inhibiting IKKβ expression [7]. Besides, high glucose concentration may modulate insulin signaling by reducing Akt activity [8]. The mTOR pathway plays an important role in diabetic kidney disease [9]. IKKβ has been found as a target for insulin sensitization in type 2 diabetes mellitus [10]. Growing evidences also suggest that transcriptional factors regulating the expression of inflammatory cytokines such as c-Jun N-terminal kinase (JNK) and IKKβ could cause IR by activating tumor necrosis factor-α (TNF-α) [11, 12]. In cultured cells, TNF-α can induce IR by increasing serine phosphorylation of insulin receptor substrate-1 (IRS-1) and convert it to inhibit the activity of insulin receptor tyrosine kinase [13]. Under chronic hyperglycemia, TNF-α production is increased and its harmful effects on insulin sensitivity are found [14]. In addition, anti-inflammatory medications may have the opposite effect [10, 15], suggesting that inflammation cytokines may be involved in the development of IR.

TNF-α is produced by a variety of inflammatory cells and plays important roles in tumor development [16], inflammation [17], ischemia reperfusion [18], and critical illness [19]. The previous study has demonstrated that IR due to chronic inflammatory factors secreted by fat cells can be improved by anti-TNF-α antibodies [20]. Recent clinical trial has also revealed that anti-TNF-α antibodies can improve IR of patients with rheumatoid arthritis [21]. However, the specific underlying roles of anti-TNF-α antibody on IR and blood glucose in rats with sepsis-induced stress hyperglycemia are still poorly understood.

In this study, we established the models of sepsis-induced stress hyperglycemia in rats, and then, the model rats were treated with anti-TNF-α antibody or insulin. The blood glucose levels, serum insulin levels, serum lipopolysaccharide (LPS), C-reactive protein (CRP), inflammatory factors, and reactive oxygen species (ROS) production in liver tissue were detected, as well as IKKβ/NF-kB inflammatory pathway and Akt/mTOR insulin signaling pathway were evaluated, aiming to explore the mechanism of acute IR caused by the trauma and infection.

Materials and methods

Animal models

The healthy male Wister rats weighing 200–250 g were purchased from the Experimental Animal Center of Shandong University (Shandong, China). All the surgical procedures for animal use were approved by the University of Shandong Institutional Animal Care and Use Committee. The rats were maintained in a temperature- and light-controlled environment with free access to food and water. After 1 week of acclimatization, the sepsis-induced stress hyperglycemic rats model was constructed by cecal ligation and puncture (CLP) combined with the intraperitoneal injection of LPS. In brief, rats were fasted for 12 h prior to surgery. After ketamine anesthesia at room temperature, the abdominal cavity of the rats was opened. The terminal cecum was ligated using surgery line, and then, the intestinal wall was pierced by surgical needle. Subsequently, a little stool was extruded, and then, cecum was put into the abdominal cavity, followed by the suture of the abdominal wall. After the CLP surgery, the rats received the intraperitoneal injection of LPS (3 mg/kg) diluted with normal saline.

Study design

A total of 350 rats were randomly divided into six groups: normal control (NC) group ($n = 54$), surgical rats without antibody or insulin therapy (Cntl) group ($n = 61$), high-dose anti-TNF-α antibody therapy (TNF) group ($n = 53$), low-dose anti-TNF-α antibody therapy (Tnf) group ($n = 59$), insulin therapy (INS) group ($n = 66$), and insulin combined with low-dose anti-TNF-α antibody therapy (INS + Tnf) group ($n = 57$). After suturing the abdominal cavity, four drug treatment groups were treated as Table 1. Anti-TNF-α monoclonal antibody was purchased from infliximab (product name: Remicade, Janssen company, Xi'an, China).

Measurements of blood glucose concentration

Blood samples were obtained from the peripheral capillaries of tails at preoperative fasting, postoperative 1 h, every 2 h within 12 h, and 3–4 times daily until 7 days. Then, the blood glucose concentrations were measured by a blood glucose monitoring system of OneTouch® UltraVue™ (Johnson and Johnson, New Brunswick, NJ, USA). Hyperglycemia and hypoglycemia were defined as blood glucose >11.1 mmol/L and blood glucose <2.8 mmol/L, respectively.

Intraperitoneal glucose tolerance test (IPGTT)

The IPGTT was performed 24 h after the intraperitoneal injection of glucose (10 ml/kg, 10% w/v) in five rats of the NC, Cntl, TNF, and Tnf groups. The peripheral glucose levels from the peripheral capillaries of tails were recorded by the blood glucose monitoring system of OneTouch® UltraVue™ before intraperitoneal injection and 0.5, 1, 2, 3 h after injection. Each time point was measured two times in a row and taken the mean to reduce the error.

Table 1 Administrations of four drug treatment groups

Group	Administration	
TNF	6 mg/kg of anti-TNF-α monoclonal antibodies by the intraperitoneal injection	
Tnf	3 mg/kg of anti-TNF-α monoclonal antibodies by the intraperitoneal injection	
INS	*Step 1* 1 U/kg of regular insulin by the intraperitoneal injection	
	Step 2 Blood glucose was subsequently measured at postoperative 1, 2, 4, 6, 8, 10, and 12 h, respectively, and then 3–4 times daily. Rats were treated with different administrations according to different blood glucose as follows	
	>11.1 mmol/L at postoperative 6 h	2 U/kg of regular insulin by the intraperitoneal injection and 0.25 U/kg of insulin glargine by the subcutaneous injection in nape once daily
	7.8–11.1 mmol/L at postoperative 6 h	1 U/kg of regular insulin by the intraperitoneal injection and 0.25 U/kg of insulin glargine by the subcutaneous injection in nape once daily
	5.6–7.8 mmol/L at postoperative 6 h	0.25 U/kg of insulin glargine by the subcutaneous injection in nape once daily
	<2.8 mmol/L at any time	2–3 mL of 10% glucose (10 mL/kg) by the intraperitoneal injection immediately, followed by the continue monitoring of blood glucose
INS + Tnf	3 mg/kg of anti-TNF-α monoclonal antibodies and 1 U/kg of regular insulin by the intraperitoneal injection	

TNF-α Tumor necrosis factor-α, *TNF* high-dose anti-TNF-α antibody therapy, *Tnf* low-dose anti-TNF-α antibody therapy, *INS* insulin therapy

Hyperinsulinemic–euglycemic clamp analysis

Five rats in the NC, Cntl, TNF, and Tnf groups were anesthetized with ketamine (40 mg/kg i.p.). The analysis of euglycemic clamp was performed under non-stressful conditions with conscious rats after IPGTT. After the injection of 20% glucose, a hyperinsulinemic–euglycemic clamp was used for 50 min; meanwhile, insulin was infused at a rate of 20 mU/kg min. Plasma glucose concentration was maintained at basal concentrations by the infusion of 20% glucose at a variable rate. The glucose infusion rates (GIR) were recorded.

Enzyme-linked immune-sorbent assay (ELISA)

Serum insulin, TNF-α, IL-6, CRP, and LPS levels were measured by ELISA. At postoperative 0, 2, 6, 24, 72 h or 7 days, the abdominal cavity was opened, and then, the portal vein was exposed. Arterial blood from abdominal aortic was collected and then centrifuged at 2500 rpm for 10 min. Serum was removed and stored at −20 °C. Serum insulin, TNF-α, IL-6, CRP, and LPS concentrations were measured using the corresponding commercial kits (R&D Systems, Minneapolis, MN, USA; Luminex Corp., Austin, TX, USA; R&D Systems; Life Diagnostics, Inc., Westchester, PA, USA; R&D Systems) according to the manufacturer's instructions.

ROS detection

At postoperative 0, 2, 6 h, 1, 3, or 7 days, the abdominal cavity was opened, and then, the livers were removed and frozen in liquid nitrogen. ROS content was detected using Reactive Oxygen Species Assay Kit (KeyGen BioTECH, Nanjing, China). Briefly, 1 g liver samples were collected and washed with cleaning liquid. After centrifuged at 300*g* for 5 min, ROS content was detected using fluorescence spectrophotometer according to the manufacturer's instructions.

Western blotting

Liver tissues were homogenized at 4 °C in lysis buffer. Totally, 30 μg proteins per lane was separated by 7.5% SDS-PAGE and transferred to polyvinylidene fluoride membranes. Then, the membranes were immunoblotted with primary antibodies: anti-phospho (p)-mTOR (Invitrogen, Carlsbad, CA, USA), anti-mTOR (Invitrogen), and anti-p-S473-Akt (Cell Signaling Technology, Danvers, MA, USA), anti-Akt (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-NF-κB-P65 (Invitrogen), and anti-SOCS-3 (Cell Signaling Technology), and anti-IKKß (Santa Cruz Biotechnology) at 4 °C overnight, followed by the incubation of Horseradish peroxidase-conjugated secondary antibody (Pierce Chemical Co., Rockford, IL, USA). Related expression was quantified using LabWorks 4.5 software (BioImaging Systems, Upland, CA, USA) and calculated according to the β-actin reference bands.

Statistical analysis

Data were presented as mean ± standard deviation (SD) and analyzed by one-way ANOVA followed by Tukey–Kramer Multiple Comparisons Test or Fisher exact test. Kaplan–Meier curve of rats was analyzed and multivariate cox regression analysis was used to estimate the

prognostic factors of survival. All tests were two-tailed and computed using SPSS v13.0 (SPSS Inc., Chicago, IL). *P* value <0.05 was considered statistically significant.

Results

Effects of anti-TNF-α antibody or insulin therapy on blood glucose level and survival in rats with sepsis-induced stress hyperglycemia

As shown in Fig. 1a, blood glucose levels raised in rats at 1 h after surgery, reached the peak at 2–4 h, and gradually reduced from 6 h. Glucose levels of rats were always higher than pre-operation. There was no significant difference of blood glucose concentrations at the same time among non-insulin-treated groups (Cntl, TNF, tnf) or insulin-treated groups (INS, INS + Tnf). The results showed that the incidences of hyperglycemia in the insulin-treated groups were significantly lower than other groups ($P < 0.05$, Table 2). The incidences of hypoglycemia and 7-day mortality in each group were also not significantly different (Table 2). In spite of no statistically significant, 7-day mortality in the TNF-α group was slightly lower than other groups (Table 2). Further Kaplan–Meier analysis revealed the highest cumulative survival probability rate in the TNF group and the lowest cumulative survival probability in the INS group, while no difference was found among these groups ($P = 0.142$, Fig. 1b). Multivariate cox regression analysis showed that both insulin-treatment and anti-TNF-α antibody treatment were not related to survival, while both hyperglycemia and hypoglycemia accidents were the prognostic factors of survival [hazard ratio (HR): 2.306, 95% confidence interval (CI): 5.085–19.796, $P < 0.001$, and HR: 2.594, 95% CI 7.116–25.161, $P < 0.001$, Table 3].

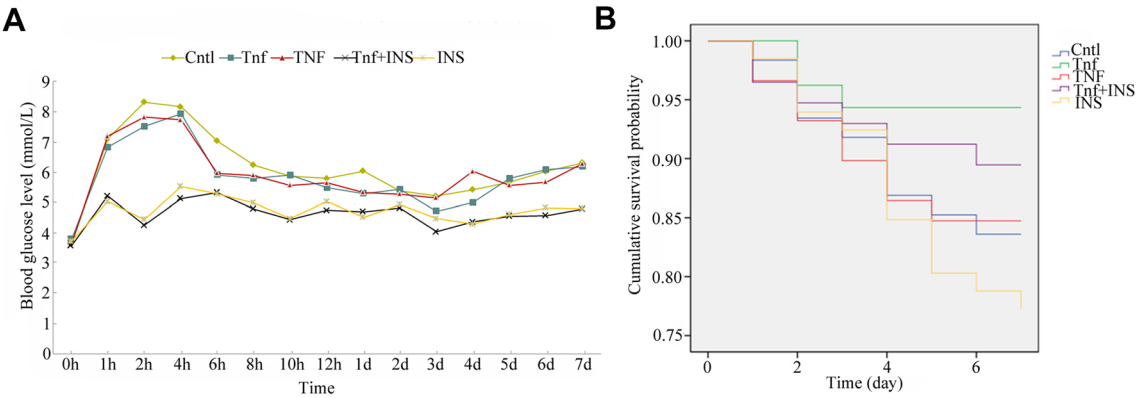

Fig. 1 Anti-TNF-α antibody or insulin therapy had no influence on blood glucose level and survival in rats with sepsis-induced stress hyperglycemia. **a** Blood glucose levels in the Cntl, Tnf, TNF, INS, and Tnf + INS groups at postoperative 0, 2, 6, 8, 12 h, 1, 2, 3, 4, 5, 6, and 7 days; **b** cumulative survival probability in the Cntl, Tnf, TNF, INS, and Tnf + INS groups by Kaplan–Meier analysis. *TNF*-α Tumor necrosis factor-α, *Cntl group* surgical rats without antibody or insulin therapy group, *TNF group* high-dose anti-TNF-α antibody therapy group, *Tnf group* low-dose anti-TNF-α antibody therapy group, *INS group* insulin therapy group, *INS + Tnf group* insulin combined with low-dose anti-TNF-α antibody therapy group

Table 2 Incidences of hypoglycemia, hyperglycemia, and 7-day mortality in the Cntl, Tnf, TNF, Tnf + INS, and INS groups

Group	Hyperglycemia incidence	Hypoglycemia incidence	Mortality
Cntl	13.11% (8/61)	13.11% (8/61)	18.03% (11/61)
TNF	7.55% (4/53)	7.55% (4/53)	5.66% (3/53)
Tnf	10.17% (6/59)	8.47% (5/59)	15.25% (9/59)
Tnf + INS	1.75% (1/57)*	15.79% (9/57)	12.28% (7/57)
INS	1.52% (1/66)*	18.18% (12/66)	24.24% (16/66)
Total	6.76% (20/296)	12.50% (37/296)	15.54% (46/296)

TNF-α Tumor necrosis factor-α, *Cntl group* surgical rats without antibody or insulin therapy group, *TNF group* high-dose anti-TNF-α antibody therapy group, *Tnf group* low-dose anti-TNF-α antibody therapy group, *INS group* insulin therapy group, *INS + Tnf group* insulin combined with low-dose anti-TNF-α antibody therapy group

* $p < 0.05$ compared with hypoglycemia incidence

Table 3 Multivariate cox regression analysis of survival of rats under different treatments

Index	HR	95% CI	*P* value
Insulin-treated or not	0.574	0.903–3.489	0.096
Anti-TNF-α-treated or not	−0.118	0.641–1.230	0.476
Hyperglycemia accident	2.306	5.085–19.796	**<0.001**
Hypoglycemia accident	2.594	7.116–25.161	**<0.001**

Bold represents statistical significance

HR Hazard ratio, *CI* confidence interval, *TNF-α* tumor necrosis factor-α

Effect of anti-TNF-α antibody on glucose tolerance and IR in rats with sepsis-induced stress hyperglycemia

IPGTT analysis was performed to examine the effect of anti-TNF-α antibody on glucose control. As shown in Fig. 2a, there was a rise in blood glucose level, with the peak occurring at 60 min after glucose injection in each group. Compared with the NC group, blood glucose levels in rats of each surgery group were significantly increased ($P < 0.01$). In addition, compared with the Cntl group, blood glucose levels were obviously decreased in the TNF group at 60, 120, and 180 min after surgery ($P < 0.05$), while only slight reduction of blood glucose levels was found without statistical difference in the Tnf group (Fig. 2a). Moreover, ELISA results revealed that the serum insulin level showed a peak at postoperative 6 h in all these three groups (Fig. 2b). The serum insulin concentration in the TNF (3.02 ± 0.48 ng/mL) and Tnf groups (3.36 ± 0.64 ng/mL) was significantly lower than that in the Cntl group (4.53 ± 1.02 ng/mL, $P < 0.05$, Fig. 2b) at postoperative 6 h. The hyperinsulinemic–euglycemic clamp analysis revealed that the GIR level was higher in the NC group (26.80 ± 2.54 mg/kg min) than that in the Cntl (11.96 ± 2.85 mg/kg min), Tnf (15.94 ± 3.17 mg/kg min), and TNF (19.76 ± 3.48 mg/kg/min, $P < 0.01$, Fig. 2c) groups. Besides, the GIR level was significantly elevated in the Tnf and TNF groups compared with the Cntl group ($P < 0.05$, Fig. 2c).

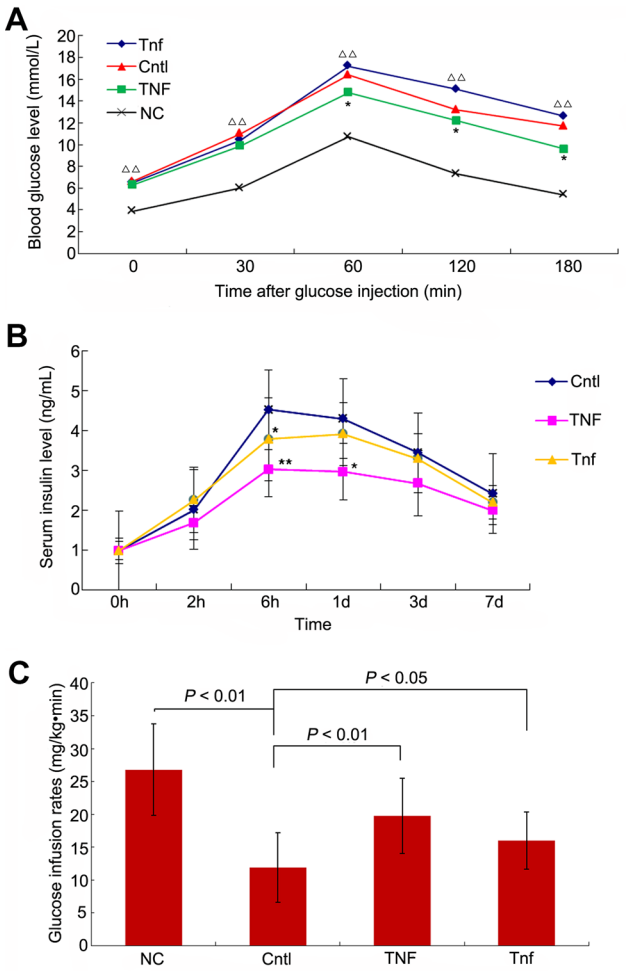

Fig. 2 Anti-TNF-α antibody could inhibit glucose tolerance and improve insulin resistance in rats with sepsis-induced stress hyperglycemia. **a** Blood glucose levels in the NC, Cntl, TNF, and Tnf groups using intraperitoneal glucose tolerance test; **b** serum insulin concentrations in the Cntl, TNF, and Tnf groups at postoperative 0, 2, 6 h, 1, 3, and 7 days; **c** glucose infusion rate (GIR) in the NC, Cntl, TNF, and Tnf groups by hyperinsulinemic–euglycemic clamp. *$P < 0.05$, **$P < 0.01$ vs. the Cntl group, $^{\triangle}P < 0.01$ vs. the NC group. *TNF-α* Tumor necrosis factor-α, *NC group* normal control group, *Cntl group* surgical rats without antibody or insulin therapy group, *TNF group* high-dose anti-TNF-α antibody therapy group, *Tnf group* low-dose anti-TNF-α antibody therapy group

Effect of anti-TNF-α antibody or insulin therapy on the levels of LPS, TNF-α, IL-6, CRP, and ROS in rats with sepsis-induced stress hyperglycemia

The levels of serum LPS, TNF-α, and CRP elevated at postoperative 2 h and reached to a peak at postoperative 1 day, and then slightly decreased in the following time (Fig. 3a–c). Compared with the Cntl group, serum LPS level was significantly decreased in four drug treatment groups at postoperative 1 day ($P < 0.05$), in the TNF, INS, and Tnf + INS groups at postoperative 3 days ($P < 0.05$), and in the TNF and Tnf + INS groups at postoperative 7 days ($P < 0.05$), respectively (Fig. 3a). Serum TNF-α levels in the TNF and Tnf + INS groups at postoperative 2 h, in the TNF group at postoperative 6 h, and in four drug treatment groups at postoperative 1, 3, and 7 days, respectively, were significantly lower than those in the Cntl group ($P < 0.05$, Fig. 3b). Serum CRP levels in the TNF group at postoperative 6 h, in the TNF and Tnf + INS groups at postoperative 1 day, in four drug treatment groups at postoperative 3 days, and in the INS, TNF, and Tnf + INS groups at postoperative 7 days, respectively, were significantly lower than those in the Cntl group ($P < 0.05$, Fig. 3c). The levels of serum

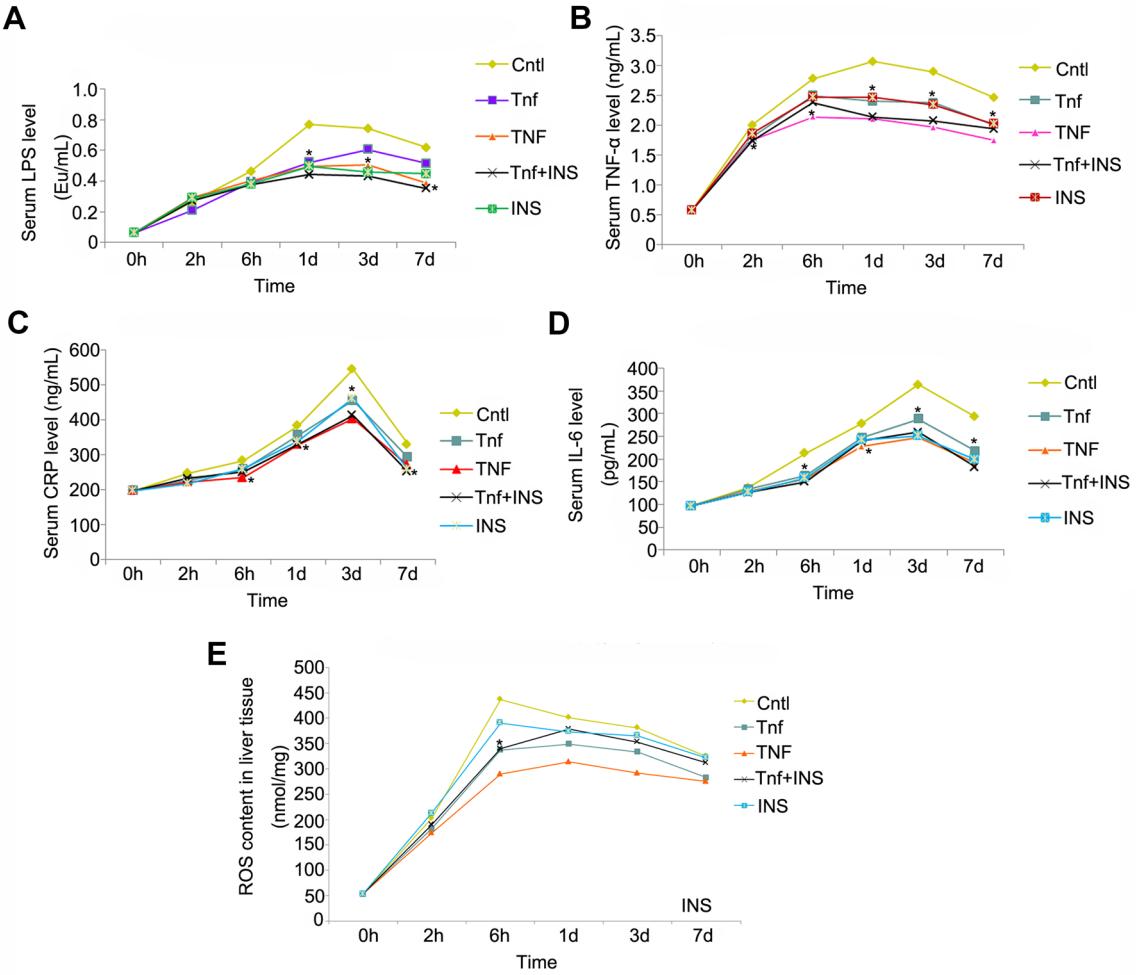

Fig. 3 Anti-TNF-α antibody or insulin therapy could inhibit the levels of LPS, TNF-α, CRP, IL-6, and ROS in rats with sepsis-induced stress hyperglycemia. The levels of LPS, TNF-α, CRP, IL-6, and ROS GIR in the Cntl, TNF, Tnf, INS, and Tnf + INS groups at postoperative 0, 2, 6 h, 1, 3, and 7 days using enzyme-linked immune-sorbent assay. *$P < 0.05$ vs. the Cntl group. *LPS* Lipopolysaccharide, *TNF-α* tumor necrosis factor-α, *CRP* C-reactive protein, *IL-6* interleukin-6, *ROS* reactive oxygen species, *Cntl group* surgical rats without antibody or insulin therapy group, *TNF group* high-dose anti-TNF-α antibody therapy group, *Tnf group* low-dose anti-TNF-α antibody therapy group, *INS group* insulin therapy group, *INS + Tnf group* insulin combined with low-dose anti-TNF-α antibody therapy group

IL-6 elevated at postoperative 2 h and approved to a peak at postoperative 3 days, and then slightly decreased in the following time. Serum IL-6 levels in four drug treatment groups at postoperative 6 h, 3, and 7 days, in the TNF and Tnf + INS groups at postoperative 1 day, respectively, were significantly lower than those in the Cntl group ($P < 0.05$, Fig. 3d). ROS content in liver tissues elevated at postoperative 2 h and approved to a peak at postoperative 6 h, and then slightly decreased in the following time. Compared with the Cntl group, ROS content was reduced in the Tnf, TNF, and Tnf + INS groups at postoperative 6 h ($P < 0.05$, Fig. 3e).

Effect of anti-TNF-α antibody or insulin therapy on AKt/mTOR and IKKβ/NF-κB signaling pathway in rats with sepsis-induced stress hyperglycemia

Western blotting analysis revealed that p-Akt/Akt ratio was significantly increased in the Cntl group compared to the NC group ($P < 0.001$), while obviously reduced in Tnf ($P < 0.05$), TNF ($P < 0.01$) and Tnf + INS ($P < 0.05$) groups compared with the Cntl group (Fig. 4a). Likewise, p-mTOR/mTOR ratio was remarkably higher in the Ctnl group than that in the NC group, while lower in the TNF ($P < 0.05$), Tnf + INS ($P < 0.01$), and INS ($P < 0.05$)

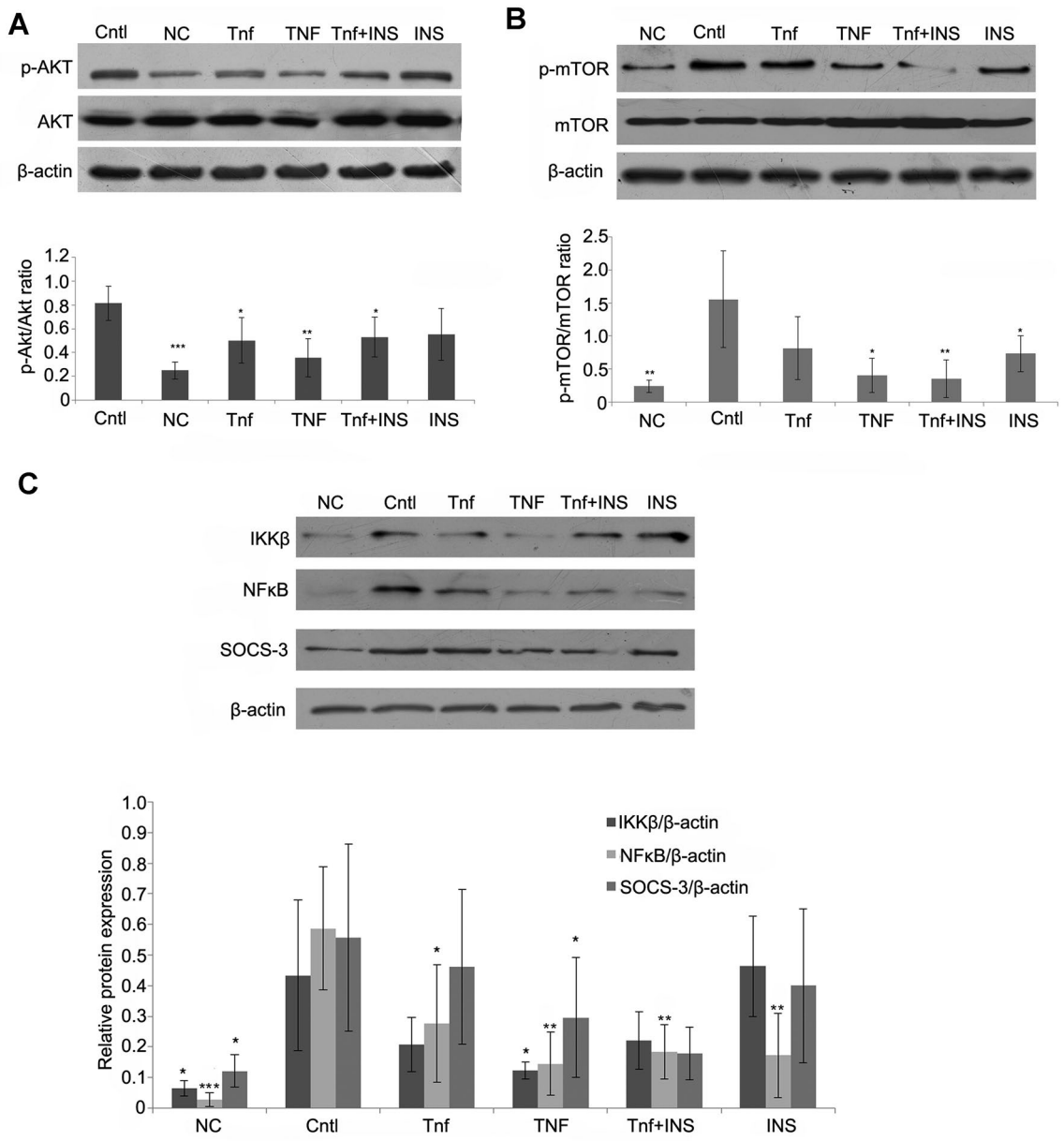

Fig. 4 Anti-TNF-α antibody or insulin therapy could inhibit AKt/mTOR and IKKβ/NF-κB signaling pathways in rats with sepsis-induced stress hyperglycemia. **a** Expression of Akt, p-Akt, and analysis of p-Akt/Akt ratio in the NC, Cntl, TNF, Tnf, INS, and Tnf + INS groups using western blotting; **b** expression of mTOR, p-mTOR, and analysis of p-mTOR/mTOR ratio in the NC, Cntl, TNF, Tnf, INS, and Tnf + INS groups using western blotting; **c** expression of NF-κB, IKKβ, and SOCS-3 in the NC, Cntl, TNF, Tnf, INS, and Tnf + INS groups using western blotting; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. the Cntl group. *TNF-α* Tumor necrosis factor-α, *p-Akt* phospho-Akt, *Akt* serine/threonine kinase, *mTOR* mammalian target of rapamycin, *NF-κB* nuclear factor-κB, *IKKβ* I kappa beta kinase, *SOCS-3* suppressor of cytokine signaling, *NC group* normal control group, *Cntl group* surgical rats without antibody or insulin therapy group, *TNF group* high-dose anti-TNF-α antibody therapy group, *Tnf group* low-dose anti-TNF-α antibody therapy group, *INS group* insulin therapy group, *INS + Tnf group* insulin combined with low-dose anti-TNF-α antibody therapy group

groups than that in the Ctnl group (Fig. 4b). In addition, the expression levels of IKKβ, NF-κB, and SOCS-3 were obviously increased in the Cntl group compared to the NC group ($P < 0.05$). The expression levels of IKKβ and SOCS-3 were significantly reduced in the TNF group compared with the Cntl group, and NF-κB expression was remarkably lower in four drug treatment groups than that in the Cntl group ($P < 0.05$, Fig. 4c).

Discussion

As a systemic inflammatory responding to severe infections, sepsis could cause high morbidity and mortality rates [22]. The present study revealed that anti-TNF-α antibody or insulin therapy had no influence on blood glucose level and survival in rats with sepsis-induced stress hyperglycemia, while hypoglycemia or hyperglycemia accident was the prognostic factors of survival. Anti-TNF-α antibody could inhibit glucose tolerance and improve IR. In addition, anti-TNF-α antibody or insulin therapy inhibited the levels of LPS, TNF-α, CRP, IL-6, and ROS; meanwhile, AKt/mTOR and IKKβ/NF-κB signaling pathways were inhibited by anti-TNF-α antibody or insulin therapy.

Stress hyperglycemia had been known to be involved in the molecular mechanism of stress hormones, and pro-inflammatory cytokines could cause hyperglycemia through various kinds of mechanisms [2, 23]. For example, TNF-α had been found to induce IR by increasing the secretion of stress hormones [24]. Study had shown that the elevated blood TNF-α concentration could reduce glucose uptake in skeletal muscle in a rat model of zymosan-induced inflammatory response [25]. In this study, we found that after treatment with intraperitoneal injection anti-TNF-α antibody of either 6 or 3 mg/kg, the level of serum insulin was significantly lower than that in the Cntl group at postoperative 6 h. A previous study had reported that insulin sensitivity had not changed when given an infusion of 5 mg/kg body weight of recombinant-engineered human TNF-α neutralizing antibody [26]. Nevertheless, other report identified that when anti-TNF-α antibodies were infused into patients with rheumatoid arthritis, a secondary benefit of enhanced insulin sensitivity was observed [27]. Similarly, our study showed an improved IR after the treatment of anti-TNF-α antibody. These findings suggested the inhibiting effect of anti-TNF-α antibody on IR in rats with sepsis-induced stress hyperglycemia.

The previous study had suggested that IR could be induced by mTOR pathway [28]. Akt, as the upstream factor of mTOR signaling pathways, was proved to be associated with insulin signaling [8]. This study revealed that both anti-TNF-α antibody and insulin significantly down-regulated p-Akt and p-mTOR, indicating that the inhibiting effect of anti-TNF-α antibody on IR was associated with AKt/mTOR signaling pathway. Inflammation was thought to be an important driver of obesity-induced IR [29]. It had been reported that TNF-α, IL-6, CRP, LPS, and ROS were involved in the pro-inflammatory response and oxidative stress [30]. Our study found that anti-TNF-α antibody inhibited the levels of TNF-α, IL-6, CRP, LPS, and ROS, suggesting the association of the inhibition of inflammation and improved IR. Another finding of the present study was that anti-TNF-α antibody reduced the expression of NF-κB, IKKβ, and SOCS-3 in rats with sepsis-induced stress hyperglycemia. Consistently, inhibiting IKKβ/NF-κB pathway could improve IR [31]. Hyperglycemia-induced oxidative stress has the ability to activate stress-sensitive signaling pathways including NF-κB [32]. Studies have found that TNF-α could activate the IKKβ pathways [10]. Conversely, NF-κB regulated TNF-α by activating IKKβ, which indicated that TNF-α was associated with IKKβ to induce NF-κB activation in type 2 diabetes [33]. SOCS-3 belonged to insulin signaling system and functions as an insulin-induced negative regulator [34]. In the liver, TNF-α might induce the expression of SOCS-3 [35]. TNF-α-induced SOCS-3 was upregulated in the adipose tissue of obese mice and could inhibit insulin signaling [36]. These results indicated that anti-TNF-α antibody could reduce IR by inhibiting the activation of IKK-β, NF-κB, and SOCS-3 in sepsis-induced stress hyperglycemia.

However, there are still many limitations in the current study. First, although we found the relationship between anti-TNF-α antibody and sepsis-induced stress hyperglycemia, it is difficult to elucidate the interaction between them and more studies are still needed to verify these mechanisms. Second, the activation assays of NF-κB, IKKβ, and SOCS3 were not performed. Third, the hyperinsulinemic–euglycemic clamp is commonly used to distinguish between hepatic and peripheral IR. However, in this study, we only analyzed the peripheral IR using the hyperinsulinemic–euglycemic clamp. Thus, to compare the results between hepatic and peripheral IR, more investigations are required. Last but not the least, the observed difference of anti-TNF-α antibody dose in analysis of IPGTT and hyperinsulinemic–euglycemic clamp does not reveal the specific effectiveness of dose in IR. Additional studies based on different dose of anti-TNF-α antibody may be needed to further elucidate the relationship between anti-TNF-α antibody and IR in sepsis-induced stress hyperglycemia.

Conclusion

This study indicated that anti-TNF-α antibody and insulin could reduce IR by inhibiting AKt/mTOR signaling pathway as well as the expression levels of NF-κB, IKKβ, and SOCS-3 in rats with sepsis-induced stress hyperglycemia.

Compliance with ethical standards

Funding This study was supported by China Postdoctoral Science Foundation (No. 20080441294).

Conflict of interest The author declares that they have no conflict of interest.

Ethical approval All the surgical procedures for animal use were approved by the University of Shandong Institutional Animal Care and Use Committee.

Informed consent No informed consent needed.

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