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Impact of β -Adrenoceptor Blockade on Systemic Inflammation and Coagulation Disturbances in Rats with Acute Traumatic Coagulopathy

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Background: Sympathetic hyperactivity occurs early in acute traumatic coagulopathy (ATC) and is closely related to its development. β -adrenoceptor antagonists are known to alleviate adverse sympathetic effects and improve outcome in various diseases. We investigated whether β -blockers have protective effects against inflammation and endothelial and hemostatic disorders in ATC.

Material/Methods: ATC was induced in male Sprague-Dawley rats by trauma and hemorrhagic shock. Rats were randomly assigned to the sham, ATCC (ATC control), and ATCB (ATC with beta-adrenoceptor blockade) groups. Rats were injected intraperitoneally with propranolol or vehicle at baseline. Heart rate variability (HRV) and markers of inflammation, coagulation, and endothelial activation were measured, and Western blotting analysis of nuclear factor (NF)- κ B was done after shock. Separate ATCC and ATCB groups were observed to compare overall mortality.

Results: HRV showed enhanced sympathetic tone in the ATCC group, which was reversed by propranolol. Propranolol attenuated the induction of pro-inflammatory cytokines TNF- α and IL-6, as well as fibrinolysis markers plasmin antiplasmin complex and tissue-type plasminogen activator. The increased serum syndecan-1 and soluble thrombomodulin were inhibited by propranolol, and the NF- κ B expression was also decreased by propranolol pretreatment. But propranolol did not alter overall mortality in rats with ATC after shock.

Conclusions: Beta-adrenoceptor blockade can alleviate sympathetic hyperactivity and exert anti-inflammatory, anti-fibrinolysis, and endothelial protective effects, confirming its pivotal role in the pathogenesis of ATC. Its mechanism in ATC should be explored further.

MeSH Keywords: Hemorrhage • Propranolol • Receptors, Adrenergic, beta

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Background

Acute traumatic coagulopathy (ATC) is a recently defined endogenous coagulopathy [1] that is mainly driven by the combination of tissue injury and hypoperfusion [2]. Several studies showed that about one-third of severe trauma patients present to the emergency room with ATC [1,3,4], which is primarily characterized by hypocoagulability [3] and hyperfibrinolysis [5] and is independently associated with higher transfusion requirement [6], multiorgan failure, and mortality [3]. Conversely, timely treatment of this disease efficiently decreases blood transfusion and improves outcome [4].

Despite significant progress in the prophylaxis and treatment of ATC (e.g., the application of “hemostatic resuscitation [7]” and the early use of tranexamic acid [8] in traumatic emergency medicine), the precise pathogenesis of ATC is still not completely understood. In addition to the activation of protein C serving as its major trigger [9], increasing proofs demonstrated that the principle drivers of ATC include tissue hypoperfusion [2], post-traumatic inflammation [10], and the activation of the neurohumoral system [11,12], which are tightly interlinked and may in turn become maladapted, leading to poor outcome. More importantly, Johansson et al. recently showed that ATC may be induced by circulating catecholamine surge resulting from severe trauma and massive hemorrhage, which is also associated with systemic inflammation [12], endothelial damage [11], and mortality [13]. Indeed, the sympathetic nervous system (SNS) plays the pivotal role in modulating acute counter-regulatory stress responses following trauma and shock via adrenergic receptors [14]. Excess adrenergic stimulation under pathological conditions, however, may disturb endogenous homeostasis and result in multisystem disorder [14]. Similarly, sympathetic over-excitation may occur in ATC and lead to autonomic dysfunction [15].

Increasing evidence shows that SNS evidently affects systemic immune function, manifested by the impact of catecholamine on immune function through adrenergic receptor-mediated pathways [14,16]. Preclinical and clinical studies have documented that decatecholaminization via nonspecific β -blockers such as propranolol can improve clinical outcome [17] and alleviate partial adverse sympathetic effects, particularly attenuating the imbalanced systemic inflammation in various diseases [16,18–21]. Numerous studies have revealed bidirectional interactions between inflammatory response and coagulation cascade following severe traumatic injury [10]. Moreover, our previous study indicated that sympathetic hyperactivity may partly contribute to coagulation disturbance in ATC [15], but the precise role of adrenergic receptor subtypes, particularly β -adrenergic receptor, in this condition remains largely unknown. Given this background, we hypothesized that beta-blockers might directly affect hemostatic function in ATC in

analogy to acute inflammatory responses in disparate diseases [19–22]. We conducted this study to investigate the effect of β -adrenoceptor blockade on systemic inflammation, endothelial damage, and hemostatic dysfunction in a rat model of ATC.

Material and Methods

Animals

This study was approved by the Animal Care and Use Committee of Jinling Hospital, Second Military Medical University, and was performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Male Sprague-Dawley rats (weighing 330 ± 20 g) were purchased from the Animal Center of Second Military Medical University, and all rats received humane care. Animals were housed in standard conditions with food and water *ad libitum*.

Experimental protocols

Rats were anesthetized intraperitoneally with urethane (1.2 g/kg, Sigma Chemical Co., St. Louis, MO, USA). The disappearance of pedal reflex indicated adequate anesthesia, and operating tables were heated to maintain core temperature at normal range. Tracheal cannulation was implemented to facilitate respiration. The left femoral artery and vein were cannulated with polyethylene tubing (PE-50, Becton-Dickinson, Sparks, MD) to detect arterial pressure and establish vascular access. The arterial catheter was coupled to a biological function experimental system (BL-420F, Tme Technology Co., Ltd, Chengdu, China) for the measurement of heart rate (HR) and mean arterial pressure (MAP). Animals were left in stabilization for 10 min to acquire baseline parameters after anesthesia and catheterization. No resuscitation was given and anesthetic was administered as required throughout the experiment.

The development of a rat model of ATC was performed with a modified method previously described by Frith [3]. A sterile 6-cm laparotomy was done and then closed in 1 layer with surgical clips to imitate tissue injury. To closely mimic clinical conditions, rats also received bilateral femur fractures to produce higher injury severity. Hemorrhagic shock was induced by withdrawing blood to achieve a targeted MAP of 35–40 mmHg. After removal of approximately 40% of rat blood volume, this targeted MAP was maintained over the 1-h shock period by repeatedly withdrawing additional aliquots of blood.

Rats were randomly assigned to the sham, ATCC (ATC control), or ATCB (ATC with beta-adrenoceptor blockade) groups. The sham group received the anesthesia and catheterization procedure but without trauma and shock. To evaluate the effects of the nonspecific β -adrenoceptor antagonist in ATC, rats in

the ATCB group were injected intraperitoneally with propranolol (7.5 mg/kg, Sigma-Aldrich, USA) at baseline, which was dissolved in sterile and endotoxin-free saline. In our preliminary experiments, this dose of propranolol was administered intraperitoneally to anesthetized normal rats, contributing to ~15% decrease in heart rate without alteration of blood pressure (data not shown). The literature shows that this dose of propranolol is effective enough to block β -adrenergic activity in rats, with a half-time of 4~5 h [23]. In the sham and ATCC groups, rats received the same amount of vehicle solution at baseline.

To eliminate the impact of anemia, rats (n=10 in the ATCB and ATCC groups and n=5 in the sham group, all at every timepoint) were euthanized (exsanguination with an overdose of urethane) at baseline, and at 0 h, 1 h, and 2 h after shock to obtain blood samples. For the Western blotting analysis of NF- κ B activation, liver tissue was obtained from the 3 groups at 2 h after shock (n=4 per group). After the shock period, separate ATCC and ATCB groups (n=20 per group) were observed until death to compare overall mortality between 2 groups. In addition, heart rate variability (HRV) at every timepoint was determined in the sham, ATCC, and ATCB groups (n=8 per group).

Assessment of autonomic function

Under urethane anesthesia, HRV analysis in rats was conducted according to the method previously described by Rhoden et al. [24]. The electrocardiogram (ECG) and signals from the 5-min observation period were recorded by bipolar limb lead II and were handled with specialized HRV software (Chart 5.4.2, AD Instrument, Australia) to filter bearing premature and artifacts. Standard deviation of normal R-R intervals (SDNN), a time-domain parameter of HRV, was measured by calculation of the standard deviation of R-R intervals from normal sinus beats [25].

Blood sample measurements

The level of hemoglobin in arterial blood was measured by the Jinling Hospital Core Laboratory. Blood samples were collected in EDTA tubes and centrifuged at 3500 rpm for 10 min, and the supernatant plasma was stored at -70°C until planned assessment. The plasma was used to determine prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen levels by an automated blood coagulation analyzer (Sysmex CA-1500, Kobe, Japan). The following markers were detected by enzyme-linked immunosorbent assay (ELISA) according to the manufacturers' instructions: soluble thrombomodulin (sTM) (Cusabio Biotech, Wuhan, CHN), TNF- α (R&D Systems, Minneapolis, MN), IL-6 (R&D Systems, Minneapolis, MN), thrombin anti-thrombin (TAT) (Diagnostica Stago, Roche, Almere, NL), activated protein C (aPC) (SANGON Biological

Engineering, Shanghai, CHN), plasmin-antiplasmin complex (PAP) (Cusabio Biotech, Wuhan, CHN), D-dimer (USCN Life Science, Wuhan, CHN), tissue type plasminogen activator (tPA) (Cusabio Biotech, Wuhan, CHN), plasminogen activator inhibitor 1 (PAI-1) (Diagnostica Stago, Roche, Almere, NL), and syndecan-1 (Bluegene biotech, Shanghai, CHN).

Western blotting analysis

Rats in the 3 groups were euthanized 2 h after shock, and rat livers were immediately snap-frozen in liquid nitrogen (n=4 per group). Nuclear and cytoplasmic proteins from snap-frozen liver tissues were isolated as previously described [26]. Then, proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The membranes were incubated with primary antibody against NF- κ B p65 (1:1000; NeoMarkers; Thermo Fisher Scientific, Fremont, CA). Beta-actin (1:5000; NeoMarkers; Thermo Fisher Scientific, Fremont, CA) was chosen as the internal standard. After incubation with horseradish peroxidase-conjugated secondary antibody (1:2000; Jackson ImmunoResearch, West Grove, PA) for 1 h at room temperature, blots were subsequently developed using an enhanced chemiluminescence detection kit (Amersham, Buckinghamshire, UK) and exposed on Hyperfilm ECL.

Statistical analysis

Data were expressed as the mean \pm standard deviation (SD). Statistical analyses were performed using SPSS 17.0 software (SPSS Inc., USA). After being analyzed by homogeneity test for variance, NF- κ B activity data were analyzed using one-way ANOVA and multiple timepoints data were assessed by two-way ANOVA. Significant results were then analyzed post hoc using the Bonferroni test. The mortality was analyzed by the log-rank test and Kaplan-Meier method. A *P*-value <0.05 was considered significant.

Results

Baseline characteristics and changes in HR, MAP, hemoglobin and fibrinogen

We found no significant group differences in bladder temperature and respiratory rate (data not shown). The mean blood loss during experiment in the ATCC group was 9.5 ± 0.8 ml and calculated as an average blood loss of $39.7 \pm 0.7\%$; both were similar to those of the ATCB group (9.7 ± 0.6 ml, $40.3 \pm 0.5\%$; *P* >0.05). As demonstrated in Figure 1, basal MAP, hemoglobin, and fibrinogen were all similar among the 3 groups, whereas propranolol pretreatment evidently reduced HR in the ATCB group compared to the ATCC and sham groups in the experiment (*P* <0.01 ,

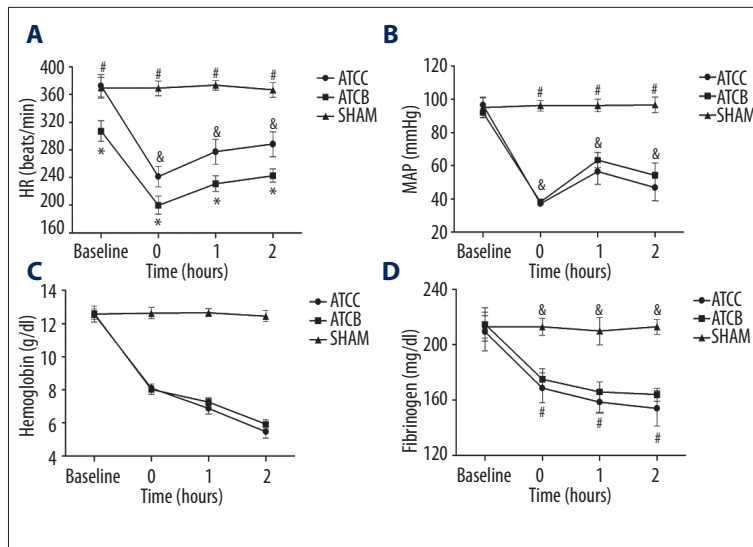


Figure 1. Effects of propranolol on (A) HR, (B) MAP, (C) hemoglobin, and (D) fibrinogen. SHAM (sham group), ATCC (ATC control group), ATCB (propranolol-treated ATC model group). Values are expressed as mean \pm SD. * $P < 0.01$, ATCC vs. ATCB; & $P < 0.01$, ATCC vs. SHAM; # $P < 0.01$ SHAM vs. ATCB.

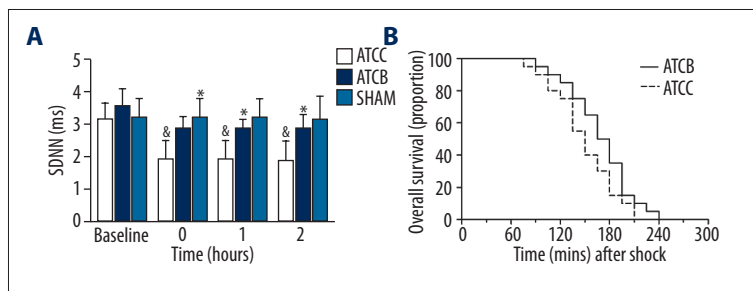


Figure 2. Effects of propranolol on (A) standard deviation of normal R-R intervals (SDNN) and (B) overall survival. ATCC (ATC control group), ATCB (propranolol-treated ATC model group). Values are expressed as mean \pm SD. * $P < 0.01$, ATCC vs. ATCB; & $P < 0.01$, ATCC vs. SHAM.

Figure 1A). Heart rate in the ATCC and ATCB groups increased after shock, indicating that propranolol pretreatment still preserved the heart rate response to trauma or shock. Following the shock period, MAP in the ATCB and ATCC groups increased at 1 h and then fell at 2 h, and MAP levels were similar between the ATCB and ATCC groups ($P > 0.05$, Figure 1B). Compared to the sham rats, there were evident decreases in the concentrations of hemoglobin and fibrinogen in the ATCB and ATCC groups after shock ($P < 0.05$, Figure 1C, 1D). No evident differences were found in hemoglobin and fibrinogen levels between the ATCB and ATCC groups ($P > 0.05$).

Effects of propranolol on autonomic function and overall survival

SDNN, a time-domain parameter of HRV reflecting total cardiac variability, was measured and results are shown in Figure 2A. Baseline SDNN was similar in the 3 groups. SDNN showed significant decrease after shock in the ATCC group compared to the sham group ($P < 0.01$). This decrease was evidently prevented in propranolol-treated ATC rats, manifested by higher SDNN in the ATCB group ($P < 0.01$). Moreover, SDNN was similar between the ATCB and sham groups, confirming that propranolol pretreatment significantly preserved parasympathetic tone and improved total variability. As shown in Figure 2B, propranolol pretreatment

did not alter overall survival after shock in the ATCB group compared to the ATCC group ($P > 0.05$), and the median time to death after shock in the ATCC and ATCB groups were 150 min and 172.5 min, respectively. Because there was no mortality in the sham rats, the overall survival of the sham group is not shown.

Effects of propranolol on serum levels of TNF- α and IL-6

As indicated in Figure 3, basal levels of TNF- α and IL-6 were similar among the 3 groups. Compared to the sham rats, tissue trauma and hypoperfusion resulted in obvious increases in serum TNF- α and IL-6 in the ATCB and ATCC group, which were persistently elevated after shock. After the shock period, the serum TNF- α level was clearly lower in the propranolol-treated rats compared with ATC control rats at 0 h, 1 h, and 2 h after shock ($P < 0.01$, $P < 0.05$, $P < 0.05$ respectively; Figure 3A). Compared to the ATCC group, propranolol evidently reduced the serum IL-6 after shock in the ATCB group ($P < 0.05$, Figure 3B).

Effects of propranolol on conventional coagulation parameters

There was no obvious change in rectal temperature of rats in the 3 groups in the experiments. The effects of propranolol on the conventional coagulation parameters, including PT and

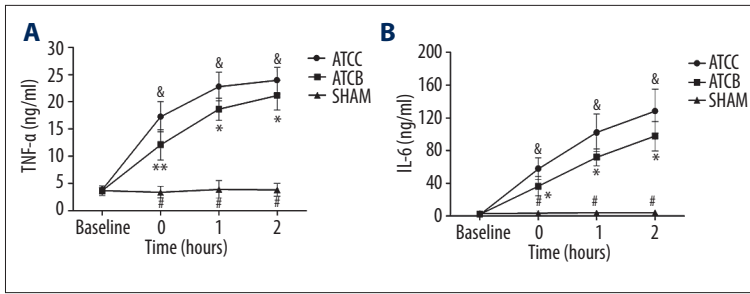


Figure 3. Effects of propranolol on concentrations of (A) tumor necrosis factor- α (TNF- α) and (B) interleukine-6 (IL-6) in serum. SHAM (sham group), ATCC (ATC control group), ATCB (propranolol-treated ATC model group). Values are expressed as mean \pm SD. * $P < 0.05$, ATCC vs. ATCB; ** $P < 0.01$, ATCC vs. ATCB; & $P < 0.01$, ATCC vs. SHAM; # $P < 0.01$, SHAM vs. ATCB.

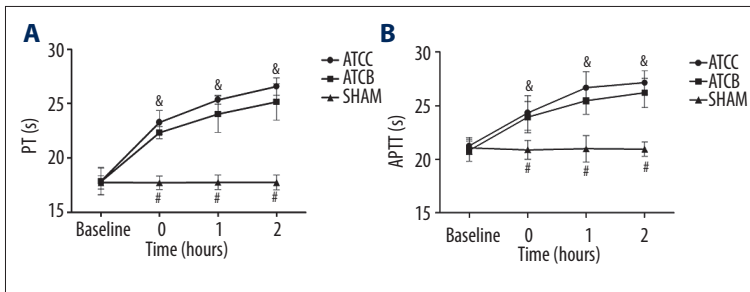


Figure 4. Effects of propranolol on (A) prothrombin time (PT) and (B) activated partial thromboplastin time (APTT). SHAM (sham group), ATCC (ATC control group), ATCB (propranolol-treated ATC model group). Values are expressed as mean \pm SD. & $P < 0.01$, ATCC vs. SHAM; # $P < 0.01$, SHAM vs. ATCB.

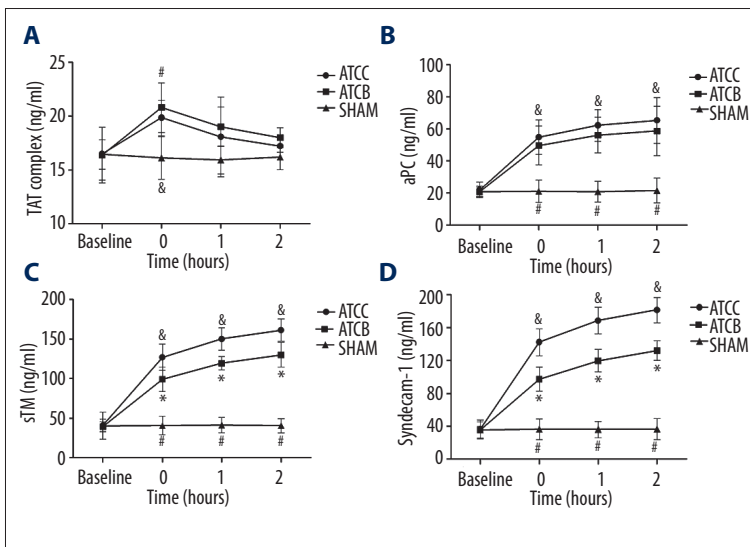


Figure 5. Effects of propranolol on concentrations of (A) thrombin-antithrombin complex (TAT), (B) activated protein C (aPC), (C) soluble thrombomodulin (sTM), and (D) syndecan-1 in serum. SHAM (sham group), ATCC (ATC control group), and ATCB (propranolol-treated ATC model group). Values are expressed as mean \pm SD. * $P < 0.01$, ATCC vs. ATCB; & $P < 0.01$, ATCC vs. SHAM; # $P < 0.01$, SHAM vs. ATCB.

APTT, are indicated in Figure 4. Basal PT and APTT levels were comparable in the 3 groups ($P > 0.05$). Compared to the sham group, the ATCB and ATCC groups had significantly higher PT and APTT levels after shock ($P < 0.01$), indicating the hypocoagulability state in ATC. However, propranolol did not change the PT and APTT levels in the ATCB group compared to the ATCC group ($P > 0.05$, Figure 4A, 4B).

Effects of propranolol on coagulation, anticoagulation, and endothelial injury parameters

To determine the effects of β -blocker on coagulation, as well as anticoagulant and endothelial activation in ATC, we measured

the serum levels of TAT, aPC, sTM, and syndecan-1 (Figure 5). These markers at baseline demonstrated no obvious group differences. Compared to the sham group, serum levels of TAT in the ATCB and ATCC groups were clearly elevated, reached a peak at 1 h after shock, and then declined ($P < 0.01$, Figure 5A). The serum TAT showed no evident difference between the ATCB and ATCC groups ($P > 0.05$). In comparison with the sham group, serum aPC, sTM, and syndecan-1 levels were obviously higher and persistently heightened after shock in the ATCB and ATCC groups ($P < 0.01$, Figure 5B–5D). Compared to the sham rats, the influence of propranolol administration on aPC did not reach statistical significance (Figure 5B). The increased serum sTM and syndecan-1 in the ATCB group after shock was

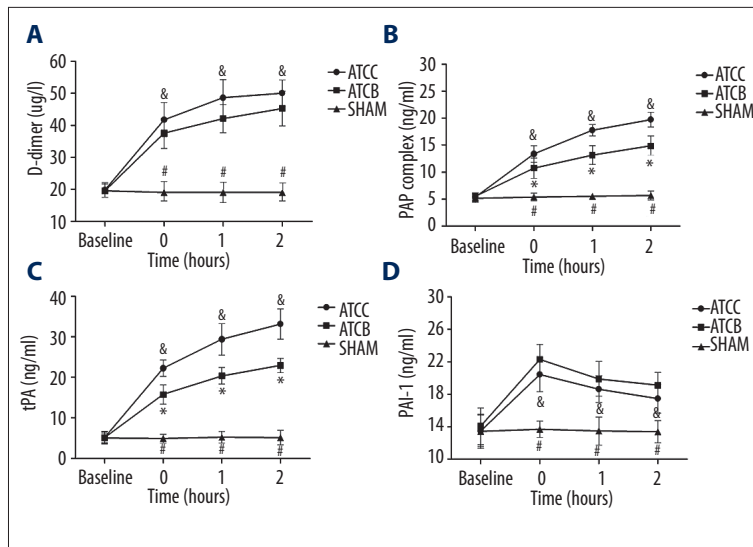


Figure 6. Effects of propranolol on concentrations of (A) D-dimer, (B) plasmin anti-plasmin (PAP) complex, (C) tissue type plasminogen activator (tPA), and (D) plasminogen activator inhibitor 1 (PAI-1) in serum. SHAM (sham group), ATCC (ATC control group), ATCB (propranolol-treated ATC model group). Values are expressed as mean \pm SD. * $P < 0.01$, ATCC vs. ATCB; & $P < 0.01$, ATCC vs. SHAM group; # $P < 0.01$, SHAM vs. ATCB.

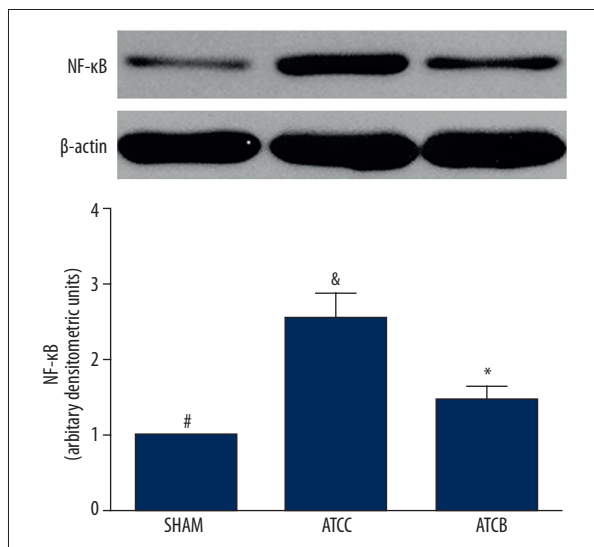


Figure 7. Effects of propranolol on nuclear factor (NF)- κ B expression in rat liver. SHAM (sham group), ATCC (ATC control group), and ATCB (propranolol-treated ATC model group). Values are expressed as mean \pm SD. * $P < 0.05$, ATCC vs. ATCB; & $P < 0.05$, ATCC vs. SHAM; # $P < 0.05$, SHAM vs. ATCB.

clearly inhibited by propranolol pretreatment, compared with those in the ATC group ($P < 0.01$, Figure 5C, 5D).

Effects of propranolol on fibrinolytic parameters

Various parameters of fibrinolysis are shown in Figure 6, and there were no evident group differences in these basal data at baseline. The combination of trauma and shock contributed to the prompt activation of the fibrinolytic system, as demonstrated by the early and persistent increase in the serum D-dimer, PAP, and tPA in the ATCB and ATCC groups ($P < 0.01$,

Figure 6A–6C). The increased serum PAP and tPA in the ATCB group was significantly inhibited by propranolol in comparison with the ATC group ($P < 0.01$). Compared with the sham rats, serum PAI-1 in the ATCB and ATCC groups promptly increased and peaked at 0 h after shock and then decreased ($P < 0.01$, Figure 6D). There was no significant difference in D-dimer and PAI-1 levels between the ATCB and ATCC groups ($P > 0.05$, Figure 6A, 6D).

Effects of propranolol on NF- κ B expression in rat liver

To elucidate the mechanism by which the propranolol pretreatment attenuated acute inflammation response, the NF- κ B activity in rat liver tissue was determined by Western blot analysis. As shown in Figure 7, Western blot analysis of liver tissue showed an obvious increase in NF- κ B expression in the ATCB and ATCC groups in comparison with the sham group ($P < 0.05$). Nevertheless, this increase was dramatically suppressed by propranolol pretreatment in the ATCB group in comparison with the ATCC group ($P < 0.05$).

Discussion

Classically, sympathetic hyperactivity might lead to end-organ dysfunction and high mortality under various pathological conditions [14–16]; therefore, maintaining appropriate sympathetic activity is essential to improve outcome [20]. Previous reports indicate that β -blockers efficiently attenuate sympathetic over-excitation and exert protective effects, such as better neurologic recovery, preserved cardiac function, decreased energy expenditure, and alleviated inflammatory dysfunction [17–22]. Consistently, blockade of β -adrenoceptor in our rat model of ATC clearly preserved total variability and suppressed sympathetic hyperactivity. In the current study, we found that acute

inflammation response induced by trauma and shock was alleviated by propranolol in rats with ATC. Furthermore, we found that tissue trauma and hypoperfusion together led to glycocalyx shedding and hyperfibrinolysis, which were clearly suppressed in propranolol-treated rats. These results suggest that β -adrenoceptor blockade can provide protection against inflammation, hyperfibrinolysis, and endothelial damage, confirming the pivotal role of β -adrenergic modulation in the pathogenesis of ATC. This mechanism might be regulated partly through the NF- κ B pathway.

In this study, the combination of trauma and shock induced early and sustained hypocoagulability and hyperfibrinolysis in rats, which closely mimics the clinical conditions of ATC in humans [3,6,9]. To avoid the influence of hemodilution on experimental results, we did not apply any resuscitation during the present study, which inevitably led to comparatively short survival time in the rats. Although overall mortality was similar between the ATCC and ATCB groups, the median time to death was increased by 15% in propranolol-treated rats. In addition, the same trends of increasing heart rate after shock in the ATCC and ATCB groups indicated that β -blockers did not suppress the heart rate response after shock. A recent clinical study also showed that preinjury β -adrenoceptor blockade did not decrease trauma patients' ability to achieve normal heart rate under fluid resuscitation [27]. Although the dose of propranolol applied in our study efficiently blocked β -adrenoceptor activity without alteration of basal blood pressure, the use of hypotensive drugs such as β -blockers in trauma and shock almost certainly causes justified safety concerns. Therefore, experiments in large-animal models of ATC should be conducted to determine the influence of different doses of β -blockers on the effects of hemostatic resuscitation.

Studies have shown that modulating the SNS via β -adrenergic receptors generates beneficial effects on inflammation markers [18,19,22]. In our study, ATC control rats showed uncontrolled production of proinflammatory cytokines, including TNF- α and IL-6, both of which were significantly inhibited by propranolol pretreatment, demonstrating the anti-inflammatory effect of β -blockers. This result is in accordance with an early study indicating that β -adrenoceptor blockade obviously decreased the expression of IL-6 in injured patients [22]. In addition, it is now widely accepted that the post-traumatic inflammation induced by tissue damage is a sterile process, which activates in a way similar to bacterial infections at both genomic and transcriptomic levels [28]. Moreover, the transcription factor NF- κ B plays the pivotal role in managing the transcription of various inflammatory genes, including TNF- α and IL-6 in inflammatory process in different types [10,29]. Previous studies demonstrated that β -adrenoceptor blockade suppresses the activation of NF- κ B [30,31]. We found that ATC control rats showed evident increase in liver NF- κ B expression after shock, which

was partially blocked by propranolol, indicating the role of β -adrenergic modulation in NF- κ B activity in ATC. But the mechanism by which β -blockers suppress the expression of NF- κ B in inflammation response still remains controversial, partly owing to the different role of the subtypes of β -adrenoceptor in inflammatory conditions, which should be clarified in our future study. We speculate that the anti-inflammatory effect of β -blockers in ATC might be generated through the downregulation of NF- κ B activity.

Although the initial characteristics of ATC are similar to disseminated intravascular coagulation (DIC) to some extent, disseminated clot formation was not found in most trauma patients in histological examination, eventually indicating the distinction between ATC and DIC [2,6]. Following trauma and shock, rats showed biphasic changes in TAT and persistent increase in aPC, indicating the characteristic hypocoagulability in ATC [1–3]. Nevertheless, propranolol did not alter serum levels of TAT and aPC or the values of PT and APTT. These findings suggest that shock is still the primary driver of ATC [3, 9], indicating that both reversal of tissue hypoperfusion and correction of coagulation disturbance are equally important in timely treatment of ATC. Hyperfibrinolysis – the over activation of primary fibrinolysis – has been considered to be integral in the pathogenesis of ATC [5,32]. A clinical study showed that fibrinolytic activation exists in most injured patients and is associated with poor outcome, which could be sensitively detected by serum PAP rather than D-dimer or thromboelastometry [32]. We observed clear increase in the serum PAP and tPA, both of which were dramatically suppressed by propranolol, suggesting the anti-fibrinolysis effect of β -blockers. Johansson et al. recently showed that circulation catecholamine directly damages endothelial cells and facilitates the release of tPA, leading to hyperfibrinolysis [11,12]. Furthermore, Stein et al. found that t-PA release derived from the vascular endothelium significantly increased after administration of β -adrenoceptor agonist [33]. In contrast, our results showed that non-selective β -blockers decrease the concentration of tPA and inhibit fibrinolytic activity, contributing to the anti-fibrinolysis effect in ATC. Since a positive relationship between sympathetic hyperactivity and Injury Severity Score (ISS) has been verified, retrospective clinical studies reported that the usage of β -blockers was associated with better clinical outcome in severe trauma patients with or without severe head injuries [17,20]. We speculate that this improvement in survival of trauma patients might be partly due to the alleviation of traumatic coagulopathy by using β -blockers.

Recent studies found that endothelial damage and glycocalyx degradation are closely involved in ATC and associated with systemic inflammation, hyperfibrinolysis, and higher mortality in trauma patients [11,12]. Our results demonstrate that serum STM and syndecan-1 levels in ATC are persistently increased

after shock, both of which were evidently reduced by propranolol, indicating the endothelial protective effect of β -blockers. Moreover, a recent study reported that TNF- α might lead to distinct glycocalyx degradation and the development of capillary leak syndrome in trauma [34]. Therefore, β -blockers might protect the endothelium partly through the inhibition of pro-inflammatory cytokine. It has been confirmed that sympatholytics might restore the sympathetic-vagal equilibrium and activate the cholinergic anti-inflammatory pathway, which could block endothelial cell activation and protect the endothelium [35,36]. The endothelial protective effect of β -adrenoceptor blockade may also be linked to the activation of the cholinergic anti-inflammatory pathway [37].

The present study has some limitations. First, since most of the detection methods applied in this study were performed in rat plasma, the coagulation dysfunction in rats may be unable to be explored promptly and globally. Considering the critical role of blood cells in the coagulation and fibrinolytic response to polytrauma and hemorrhagic shock [38], we will use a more sensitive and comprehensive approach such as thrombelastography in future research. Second, in our

hypothesis-generating research we used propranolol, a type of nonspecific β -adrenoceptor antagonist, which is more lipophilic than other beta-blockers and has better blood-brain barrier penetration. Therefore, the precise role of central and peripheral β -adrenoceptor, as well as the role of its subtypes, should be explored further.

Conclusions

We demonstrated in a rat model of ATC that preemptive β -adrenoceptor blockade can alleviate inflammation, endothelial damage, and coagulation dysfunction by attenuating the hyperadrenergic stress response in rats with ATC, the mechanism of which might be regulated partly through the NF- κ B pathway. This study preliminarily demonstrates the role of β -adrenoceptor modulation in the pathogenesis of ATC, and its precise role deserves further study.

Declaration of interest

The authors report no conflicts of interest.

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