Antithrombin III in sepsis: does it really work?

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INTRODUCTION

Excessive inflammatory reactions and, in particular, neutrophil activation have been implicated in the pathogenesis of the acute respiratory distress syndrome (ARDS) pathogenesis[1-4]. The mechanisms of such injury are thought to be related to oxygen free radical production and/or protease damage to the endothelial cells by activated neutrophils[1-9]. In endotoxemia, there is good evidence that disseminated intravascular coagulation (DIC) and acute thromboembolic events, and inappropriate inflammation, particularly neutrophil activation into tissues all contribute significantly to sepsis-induced injury of various organs[10-17]. In this context, activated leukocytes decrease their flow velocities and adhere to the vascular endothelium to emigrate to the septic focus. The subsequent release of an array of inflammatory mediators, cytotoxic enzymes, and oxygen radicals may result in reduced capillary perfusion that finally leads to the development of organ dysfunction[1,18-21].

Anti-inflammatory treatment of sepsis is still debated and inhibitors of the coagulation pathway appear effective. Antithrombin III (AT-III) is a serine protease inhibitor (serpin). The mechanism of its anti-inflammatory action is still not well understood. Antithrombin (AT) has two potential benefits in the treatment of sepsis: (1) reducing the severity of DIC; and (2) decreasing inflammation in part because of its binding to glycosaminoglycans (GAGs) on the endothelial cells and leukocytes[22,23]. It has therefore been proposed that AT supplementation might be used to control coagulation distrubances and improve the clinical consequences of the sepsis-induced inflammatory syndrome.

This article discusses the results of the experimental and main clinical studies regarding to AT supplementation in sepsis and sepsis-induced DIC.

Sepsiste antitrombin III: Tedavide yeri var mı?

Anahtar Kelimeler: Antitrombin, Sepsis, Antiinflamatuvar, Prostasiklin, Aktive lökositler, Heparin, Glikozaminoglikanlar.

Key Words: Antithrombin, Sepsis, Anti-inflammatory, Prostacyclin, Activated leukocyte, Heparin, Glycosaminoglycans.

AT-III in SEPSIS INDUCED DIC and ORGAN FAILURE

Thrombin is a multifunctional serine protease that only plays a key role in the clotting cascade by catalyzing the conversion of fibrinogen to fibrin, but it also activates a variety of cell types, including platelets and endothelial cells. Thrombin is a pro-inflammatory molecule and therefore provides a critical link between coagulation and inflammation. Thrombin signaling in endothelial cells results in increased permeability, recruitment, and rolling and firm adhesion of leukocytes through the induction of chemokins, selectins, and members of the immunoglobulin superfamily[24-26].

Cytokines, releasing of tissue factor, inflammation through thrombin-induced activation of endothelium, platelets and vascular smooth muscle in sepsis can directly damage the vascular endothelium[27,28]. Exacerbation of pro-inflammatory mediator release, endothelial cell injury, tissue factor expression and thrombin production compromise the microvascular function resulting in DIC, decreased tissue perfusion and organ failure[29-31].

Blocking DIC by suppression of thrombin generation has become an accepted target in treating the multiple organ dysfunction syndrome of severe sepsis[32]. Coagulation is initiated by the expression of tissue factor on endothelial cells and monocytes induced by endotoxin, tumor necrosis factor (TNF) and interleukin-1 (IL-1) in sepsis[33]. AT inactivates thrombin and several serine proteases including factors IXa, Xa, XIa and XIIa, kallikrein, urokinase, tissue plasminogen activator, plasmin and trypsin[34]. Therefore, natural coagulation inhibitor AT may has benefical effects on organ functions in severe sepsis and sepsis-induced DIC.

Experiments in animal models of sepsis and sepsis-induced DIC have showed that high dose of AT-III (> 250 IU kg^{-1}) has significant positive effect in preventing organ dysfunction, mortality and DIC[11,12,35-38]. The question that "Why AT-III improves DIC and decreases mortality only when given at very high doses", may be explained by the following mechanisms: (1) during sepsis, the activation of the coagulation cascade induces the generation of considerable of thrombin; and (2) inactivation of AT at the endothelial cell surface by elastase released from activated leukocytes[39].

In a large prospective study the incidence of DIC in sepsis was 16%, in severe sepsis was 18% and septic shock was 36%[40]. Studies adressed the possibility that AT supplementation could improve DIC and its clinical consequences. In these trials, AT-III significantly improved the severity or duration of DIC[41-43]. For example, Blauhut et al observed a more rapid normalization of coagulation tests, less bleeding and unchanged mortality in the AT-III treated patients compared with nontreated patients[41]. Fourier et al reported that 100 IU kg^{-1} loading dose followed by continuous infusion of 100 IU kg^{-1} day^{-1} for 4 days of AT-III significantly reduces the duration of DIC but nonsignificantly reduces mortality in septic patients[42]. In a controlled trial, long term (14 days) and high dose AT-III supplementation (activity > 120%) modulated inflammatory mediators and coagulation variables, mainly during the second week of application[43]. Therefore, following question is waiting for explanation. Is short term period of application (4 days) enough time for the coagulation inhibitor to work effectively? Therefore we need large multicentre trial to draw definitive conclusions about the indication, and benefical effects of AT-III in sepsis induced DIC.

NEUTROPHIL RECRUITMENT in SEPSIS-INDUCED INFLAMMATORY LUNG INJURY, DOES AT-III INHIBIT IT?

Neumann et al demonstrated that activation of circulating granulocytes is characterized by increased production of serine proteases and reactive oxygen metabolites, as well as elevated expression of circulating granulocyte surface macrophage antigen-1 in int-

raabdominal sepsis induced by colon ascendens stent in mice[44]. They observed that, expression of macrophage inflammatory protein-2, cytokine-induced neutrophil chemoattractant, macrophage inflammatory protein 1α and E-selectin mRNA markedly increased in the lung within 3 h following sepsis induction, whereas up-regulation of IFN-inducible protein 10, macrophage chemotactic protein-1 and P-selectin was delayed. They concluded that recruitment of preactivated neutrophils may be critical for the development of inflammatory lung injury during intraabdominal sepsis.

The recruitment of leukocytes to the site of inflammation is a series of sequential steps beginning with initial contact of leukocytes with endothelium termed tethering and rolling, which appears to be absolutely critical for subsequent adhesion and emigration of leukocytes out of the vasculature[45-47]. Leukocyte rolling is mediated by two endothelial selectins: P-selectin and E-selectin. Thrombin is a serine protease and is important in recruiting leukocytes in various inflammatory conditions including ischemia/reperfusion and sepsis[12,48]. There are a few studies to suggest that thrombin has the ability to recruit neutrophils by an early P-selectin and delayed E-selectin pathway in the initial phase of leukocyte recruitment (leukocyte rolling)[48-53]. Firm adhesion, the second phase of leukocyte recruitment, can also be induced by thrombin as a result of rapid endothelial platelet-activating factor[54].

In every inflammatory process, the vast majority of cellular events require nuclear factor κB (NF-κB) transcriptional activity[55]. NF-κB mobilization is essential for thrombin mediated VCAM-1-dependent and E-selectin-dependent neutrophil recruitment[53]. The transcriptional regulatory factor NF-κB is a central participant in modulating the expression of the immunoregulatory mediators involved in sepsis[56,57]. There is evidence that AT-III potentially blocks the activation of NF-κB, a transcription factor involved in immediate early gene activation during inflammation[58,59]. It should be remembered that NF-κB is an essential component of normal host defences and that blockade of the regulatory actions may be severely immunosuppresive. For example, mice lacking the p50 subunit of NF-κB are unable to clear *Listeria monocytogenes* effectively and are more susceptible to infection with *Streptococcus pneumoniae*[60].

AT-III can reduce sepsis-induced neutrophil recruitment into the lungs[48,49]. However, adhesion may not be the mechanism of leukocyte recruitment in sepsis-induced lung injury[38,61]. A number of investigators demonstrated that unlike most organs, in the pulmonary vasculature, leukocyte recruitment in response to lipopolysaccharide (LPS), may not be dependent upon selectins and integrins but perhaps due to physically trapping in capillaries[62-65]. Uchiba et al, have postulated that AT-III releases prostacyclin from endothelial cells, inhibits leukocyte recruitment, and thereby protects the pulmonary vasculature from injury induced by LPS in rats[61]. Woodman et al, demonstrated that neither AT-III pretreatment nor posttreatment in a feline mesentery exposed to LPS had any effect on LPS-induced selectin-dependent leukocyte rolling, adhesion, emigration, or microvascular dysfunction[65]. They explained the discrepancy between their results and Uchiba's results with the note that, because leukocyte recruitment in the mesentery is entirely dependent on the selectins and integrins, it is conceivable that nonadhesion molecule-dependent leukocyte recruitment in the lung (neutrophil trapping) is affected by AT-III via prostacyclin, an event not seen in the mesentery[61]. Also Yamashiro et al, demonstrated that AT-III treatment significantly inhibits inflammatory reactions during endotoxin-induced uveitis in rats, and concluded that AT's suppressive effects on P-selectin expression could contribute to the attenuation of leukocyte infiltration, possibly by inhibiting leukocyte rolling[66].

ANTI-INFLAMMATORY PROPERTIES AT-III

The anti-inflammatory activity of AT-III has been explained by following mechanisms: (1) thrombin inhibition that attenuates PARs-mediated IL-6, IL-8, P-selectin and PAF synthesis; (2) endothelial cell prostacyclin synthesis that diminishes platelet and neutrophil activation and attachment to endothelial cells, decreases pro-inflammatory cytokine production; and (3) binding to white cell Syndecan-4 receptor that decreases neutrophil chemotaxis, cytokine production, and chemokine receptor expression[67,68].

Interaction of AT-III with heparin-like GAGs on the endothelial cell surface has been shown to promote the release of prostacyclin from the endothelial cells in vitro and in vivo[69-72]. Wang et al demonstrated that plasma concentration of prostacyclin, in the form of stable product 6-keto prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) increases significantly at 2-20 hours after cecal ligation and perforation (CLP) in rats. Intravenous administration of 250 U kg^{-1} AT-III in rats and in the dog model of lung transplantation significantly increased the plasma 6-keto $PGF_{1\alpha}$ levels[72-74]. Although the dose of 250 U kg^{-1} of AT-III significantly prevented the endotoxin-induced pulmonary vascular injury and coagulation abnormalities in rats: the lower doses of AT-III (50-150 U kg^{-1}) prevented coagulation abnormalities, but not pulmonary injury[38].

Cytokines, such as TNF-α, IL-1β, and various inflammatory mediators derived from activated leukocytes damage endothelial cells leading to microcirculatory distrubances[1,75]. The endotoxin induced release of inflammatory mediators like IL-6, IL-8 and TNF is controlled by AT in experimental studies[76-80]. AT-III induces endothelial cell release of prostacyclin, which inhibits cytokine production and suppresses leukocyte and T-cell activation[61,69,70,81-85]. Prostacyclin inhibits synthesis of pro-inflammatory cytokines by a cyclic adenosine monophosphate-mediated process: this attenuates neutrophil activation, thereby reducing neutrophil degranulation, elastase release and toxic oxygen radical release[38,69,71]. In addition, the effect is observed primarily at supraphysiologic levels (> 200% normal levels) of AT[38]. Minamiya et al showed that AT-III reduces F-actin formation in neutrophil by binding GAGs on the neutrophil, thereby reducing neutrophil accumulation in the lung, which would in turn inhibit oxygen radical production in the lung of rat[86]. Recent studies in endotoxin-induced sepsis models demonstrate that the interaction of activated leukocytes and lymphocytes with endothelium in particular cell sticking and transmigration events, is significantly reduced after administration of therapeutic doses of AT[48,87].

AT interacts with cells via binding to GAGs, in particular those of the membran protein Syndecan-4[76]. This G protein-coupled receptor has been described to mediate focal adhesion processes and to be involved in chemotaxis and cell migration[88]. The fact that, AT's intact heparin-binding site is crucial to AT's anti-inflammatory effect has been demonstrated in different experimental settings[76,89-91]. Kaneider et al demonstrated that AT directly inhibits chemokine-stimulated migration of monocytes and lymphocytes via the effects of its heparin-binding site on cell surface Syndecan-4 by activation of protein kinase C and Rho signaling[89].

Replacement of AT improves the outcome of numerous experimental models of gram-positive and gram-negative bacterial sepsis and is useful for prevention of organ failure in animals challenged with endotoxin or bacteria and for prevention of ischemia/reperfusion injury[10-12,35,38,65,66,69,81,92-99]. Redens et al, reported that AT-III attenuated the decrease in apparent lung compliance and prevented the fall in arterial PO_2 in a sheep model of LPS-induced ARDS[100]. Okajima and coworkers, published data showing protection against pulmonary vascular injury and increase in prostacyclin in LPS-induced septic rats treatment with AT-III[61,72].

Uchiba et al, investigated the effect of AT-III on the activated leukocyte-induced pulmonary vascular injury in rats given endotoxin[61]. Intravenous administration of endotoxin to rats increased the pulmonary accumulation of neutrophils, and the subsequent pulmonary vascular injury. In their rat model of endotoxemia, IV administration of AT-III (250 U kg^{-1}) reduced leukocyte recruitment by 20% and the subsequent pulmonary vascular injury. They suggested that AT-III prevents pulmonary vascular injury by inhibiting pulmonary accumulation of neutrophils and this effect appears to be independent of the anticoagulant effect, but depends on the interaction of AT-III with heparin-like GAGs on the endothelial cell surface that promotes endothelial prostacyclin release. These observations suggest that a higher dose of AT-III is necessary to prevent endothelial cell injury than is required to inhibit coagulation abnormalities. These findings also support the notion that AT-III prevents endotoxin- induced endothelial cell injury by promoting endothelial release of prostacyclin and thus inhibiting leukocyte activation. Further, it should be noted that combined use of heparin with AT-III did not prevent the pulmonary accumulation of neutrophils or pulmonary vascular injury, probably because heparin binds AT-III in circulation, thereby preventing AT-III from interacting with heparin-like GAGs on the endothelial cell surface.

All these facts raise the possibility that AT-III prevents organ injury induced by activated leukocytes in sepsis. Sepsis is a multifunctional and multi-etiological disease in human, there could not be a single animal model which would predict the efficacy of a given drug. Thus it was recommendation for sepsis research to: (1) confirm results in independent laboratories; (2) employ different animal species; (3) include infection models with either gram-negative and gram-positive bacteria; (4) assure that the drug in question should remain effective after the initiation of the septic process; and (5) that the animal studies should provide a therapeutic rationale for the treatment of sepsis[92]. When reviewing the past studies with AT-III in animal sepsis models it becomes clear that AT-III fulfills the criteria cited above[92]. Cumulative evidence proves efficacy of AT-III in different animal species. Previous experimental studies described the possible mechanisms of efficacy of AT-III in the treatment of sepsis[61,67,68].

CLINICAL STUDIES with AT-III in HUMAN SEPSIS

Experimental studies support the use of high doses of AT concentrates, a therapeutic regimen in sepsis. However, there is no clear documentation in the clinical setting that AT might have benefical effects because of its anti-inflammatory properties. This issue was adressed in a study by Inthorn et al only[93]. They demonstrated that AT-III supplementation is associated with a significant improvement in respiratory, liver, and renal failures, with a concomitant decreases in some inflammatory markers such as selectin and intercellular adhesion molecule-1 in severe septic patients.

Baudo et al studied 120 patients with severe sepsis and postoperative complications: 55 patients had septic shock[101]. There was no differences in over-all survival between the placebo and AT-III treated patients. The probability of survival was 30% in the AT-III treated group, vs. 17% in the placebo group ($p< 0.05$). Fourrier et al, demonstrated that the duration of septic shock-induced DIC was shortened significantly by high dose of AT-III concentrates compared to that of the placebo group[42]. And they also demonstrated that mortality was reduced nonsignificantly by 44% in septic patients treated with high dose of AT-III. Ilias et al, compared the continuous infusion and intermittent bolus dose of AT-III treatment in severe sepsis[102]. The serum AT-III levels during the treatment phase (96 h) ranged from 168% to 212% in the intermittent bolus group and from 188% to 232% in the continuous infusion group.

28-days all-cause mortality was 30% (43% intermittent group: 21% continuous infusion group). The mean probability of dying according to the SAPS II was 48%. Eisele et al, compared the results of AT-III and placebo group in 42 severely septic patients[103]. They obtained nonsignificant 39% reduction in 30-days all-cause mortality in AT-III treated group. Patients treated with AT-III demonstrated a better resolution of pre-existing organ failures and lower incidence of new organ failures during the observation period. Resolution of pre-existing respiratory dysfunction in AT-III and placebo group was; 63% and 33% on days 1 and 2: 13% and 0% on day 7: and 57% and 17% on day 30, respectively. A meta-analysis of 4 placebo-controlled double blind trials (total 122 patients) documented a 22% nonsignificant decrease in the mortality rate in the AT-III treated sepsis patients[103]. These results supported the need for a phase III multicentre trial studying patients with severe sepsis (Table 1).

The KyberSept Trial was a major international phase 3 clinical placebo-controlled and double-blind study which enrolled 2314 patients[104]. It was performed to determine the efficacy of high dose AT-III in patients with severe sepsis in 211 contributing centers worldwide. In this study, the all-cause mortality at 28 days in the primary efficacy group of high dose AT was not significantly different from placebo group (38.9% vs 38.7% respectively). In the subgroup of patients who did not receive concomitant heparin during 4-day treatment phase (n= 698), the 28-day mortality was insignificantly lower in the AT-III group (37.8%), than in the placebo group (43.6%). This trend became significant after 90 days (n= 686, 44.9% for AT-III group vs 52.5% for placebo group). The authors suggested that there was some evidence to suggest a treatment benefit of AT-III in the subgroup of patients not receiving concomitant heparin. This suggestion supports the notion that AT-III has anti-inflammatory effects when used without heparin. Also experimental data, mentioned above, have shown that heparin blocks the anti-inflammatory effects of AT-III.

In the KyberSept Trial, the 28 days mortality in subgroups who had lower baseline serum AT levels (< 60%) was higher than the subgroup who had higher baseline serum AT levels (≥ 60%)[104]. The 28 days mortality in the subgroup of placebo treated patients who had baseline serum AT levels < 60% was 47.5% and who had ≥ 60% was 28.5%. Although the 28 days mortality in the subgroups of AT-III treated patients who had baseline serum AT-III levels < 60% was 46.2% and who had ≥ 60 was 29.1%. These data suggests that lower baseline serum AT-III levels correlated with higher 28 days mortality rate in severe sepsis. The mortality rate in the AT-III treated patients who did not receive concomitant heparin and had baseline serum AT-III level ≥ 60% was unclear in the KyberSept Trial.

Pettila et al evaluated the predictive value of plasma AT-III concentration in 100 critically ill patients with suspected sepsis[105]. Admission plasma AT-III concentrations was different significantly between hospital survivors and nonsurvivors (66% percentage of normal and 46% percentage of normal, respectively). However, in prediction of hospital mortality rate, the discriminative power of admission plasma AT-III concentration was poor and was not independently associated with hospital mortality rate.

Rublec et al evaluated the effects of AT-III treatment on quality of life data measured for up to 90 days during the follow-up phase of the KyberSept Trial[106]. They found that, among all sepsis survivors in the trial, there is a significant advantage on some attributes of quality of life in the AT-III subgroup of patients who did not receive heparin as compared with the corresponding placebo group. I suggest that this quality of life improvement in AT-III subgroup who did not receive concomitant heparin may be related to organ preservation effects of AT-III caused by its anti-inflammatory effects, and may be suggestive of a potential treatment benefit.

Table 1. Clinical trials of antithrombin supplementation during sepsis

Study, Author, (reference)	Study design	No of patients	Results
Baudo et al[101]	RCT AT 4000 IU loading followed by 2000 IU/12 hrs for 5 days vs. placebo	120 patients with sepsis (60 AT, 16 placebo)	Probability of survival was 30% in the AT group, vs. 17% in the placebo group ($p< 0.05$)
Eisele et al[103]	RCT AT 3000 IU loading follwed by 1500 IU/12 hrs for 5 days vs. placebo	42 patients with severe sepsis (22 placebo, 20 AT)	39% reduction in 30-days mortality in AT group, p= NS. Better resolution of existing organ failures in AT group
Fourrier et al[42]	RCT AT 90-120 IU kg^{-1} loading followed by 90-120 IU kg^{-1} day-1 for 4 days vs. placebo	35 patients with septic shock and DIC (18 placebo,17 AT)	DIC duration shortened in AT group (71% resolution after treatment vs. 33%, $p< 0.05$) 44% reduced mortality in AT group, p= NS
Inthorn et al[43]	RCT AT infused to maintain plasma AT activity 120% for 14 days vs. placebo	29 patients with severe sepsis (14 AT, 15 placebo)	Resolution of DIC in all AT group vs. none in placebo ($p< 0.05$). Improved lung function and reduced need for renal support in AT group vs. placebo, $p< 0.05$
Ilias et al[102]	Prospective, open, randomized, 2 parallel groups 6000 IU loading followed by intermittent bolus infusion of 1000 IU every 4 hrs or followed by 250 IU/hr for 4 days	33 patients with sepsis (18 continuous, 14 intermittent)	The over-all 28 days mortality was 30% (43% in intermittent bolus group; 21% in continuous infusion group) The mean probability of dying to the SAPS II was 48%
Warren et al[104] KyberSept Trial	RCT AT 6000 IU loading followed by a continuous infusion of 6000 IU per day for 4 days vs. placebo	2314 patients with severe sepsis and septic shock (1157 placebo, 1157 AT)	The 28 days mortality was 38.9% in the AT-III group and was 38.7% in the placebo group. This trend became significant after 90 days (n= 686), 44.9% for AT-III group vs. 52.5% for placebo group, p=0.03

AT: Antithrombin, RCT: Randomized-controlled trial, DIC: Disseminated intravascular coagulation, NS: Nonsignificant.

CONCLUSION

Cytokines and various inflammatory mediators derived from activated leukocytes damage endothelial cells leading to microcirculatory disturbances[75]. The number of AT binding sites per macrovascular and microvascular endothelial cell has been estimated at approximately 50.000 and up to 500.000, respectively[107]. This shows the potential importance of AT for the microcirculation.

In many animal studies, a single bolus dose of AT-III was administered before sepsis induction, and acts when inflammatory cytokine levels begin to rise[10,100,108]. In these studies, AT-III pretreatment improved organ dysfunctions and survival rate. Interestingly, in the animal model of sepsis, AT-III improved survival if given before endotoxin injection, but not after the endotoxin infusion[109]. In clinical studies, AT-III was started with a bolus dose and then continuous infusion in severe septic patients[103,104,110]. Most septic patients had decreasing cytokine levels at the time of AT-III treatment, suggesting a transition from a pro-inflammatory to hypo-inflammatory state[111]. There is a proof in clinical setting that AT-III might have benefical effects based on its anti-inflammatory properties: Inthorn et al demonstrated that AT-III supplementation in severe septic patients was associated with a significant improvement in respiratory, liver and renal dysfunction with concomitant decrease in some inflammatory markers such as selectin and intercellular adhesion molecule-1[93].

Esmon tried to explain the discrepancy between the clinical and experimental studies regarding to AT-III supplementation in sepsis as[111]:

1. Most animal studies are performed acutely in young healthy animals, where as a significant percentage of the clinical population is elderly with many secondary complications (e.g. diabetes, high blood pressure etc.),

2. In animal studies AT-III is administered before or during the very early stages of sepsis, when inflammatory cytokine levels are still rising,

3. In contrast, current treatment strategies are started when most patients are switching from a pro-inflammatory cytokine response to an anti-inflammatory response,

4. AT-III is administered at a late stage and under very different conditions in human sepsis than it is during efficacy testing in animal models.

Taken together, I suggest that, in a clinical setting, early diagnosis of septic episode or impending sepsis with immediate AT-III replacement would be of some importance to achieve beter outcome.

To my opinion, there is still need for comparative studies in order to determine the effects of AT-III on the inflammatory markers and patients survival when given in the early (pro-inflammatory) and late (hypo-inflammatory) stage of sepsis. Large multicentre trial is required to draw definitive conclusions about the indication, and benefits of AT-III in sepsis induced DIC and related organ failure. Some questions is waiting for explanation: (1) what is the therapeutic value of long term AT-III supplementation (> 4 day) in sepsis?; (2) is it possible and necessary to work anti-inflammatory effects of AT-III in septic patients? Finally, I concluded that because of AT-III has complex interactions with host coagulopatic and systemic inflammatory responses in sepsis, the impact of these interactions and therapeutic implications of administiration of AT-III need further clarification.

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