

Review

The Protective Effect of Alpha 7 Nicotinic Acetylcholine Receptor Activation on Critical Illness and Its Mechanism

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Abstract

Critical illnesses and injuries are recognized as major threats to human health, and they are usually accompanied by uncontrolled inflammation and dysfunction of immune response. The alpha 7 nicotinic acetylcholine receptor ($\alpha 7nAChR$), which is a primary receptor of cholinergic anti-inflammatory pathway (CAP), exhibits great benefits for critical ill conditions. It is composed of 5 identical $\alpha 7$ subunits that form a central pore with high permeability for calcium. This putative structure is closely associated with its functional states. Activated $\alpha 7nAChR$ exhibits extensive anti-inflammatory and immune modulatory reactions, including lowered pro-inflammatory cytokines levels, decreased expressions of chemokines as well as adhesion molecules, and altered differentiation and activation of immune cells, which are important in maintaining immune homeostasis. Well understanding of the effects and mechanisms of $\alpha 7nAChR$ will be of great value in exploring effective targets for treating critical diseases.

Key words: alpha 7 nicotinic acetylcholine receptor; critical illness; cholinergic anti-inflammatory pathway; neuroinflammation; immune function; protective effect.

Introduction

Critical illnesses are known as great threats to human health, which initiate abnormal response to insults and infections, and manifest uncontrollable inflammation and dysfunction of immune cells. Recently, the neuroendocrine-immune networks show great benefits for improving severe diseases [1]. For instance, the cholinergic anti-inflammatory pathway (CAP), which is composed of efferent vagus nerve, acetylcholine and $\alpha 7$ subunit of the nicotinic acetylcholine receptor ($\alpha 7nAChR$), has been reported to attenuate excessive inflammation in critical settings [2-7]. Activated by peripheral or central stimuli, the brain cholinergic neurons deliver the information to efferent vagus nerves which innervate peripheral organs and release acetylcholine that can inhibit the

production of pro-inflammatory cytokines by interacting with $\alpha 7nAChR$ on inflammatory cells [1, 8, 9].

$\alpha 7nAChR$ is a major receptor of CAP, and first discovered expression on neurons. It has been well-studied as a pharmacological target for neuropathological diseases, underlying a neuroprotective role of $\alpha 7nAChR$ [10]. Activation of $\alpha 7nAChR$ with GTS-21 or AQW051 significantly mitigated the severity of psychotic disorders by improving cognition, learning and working memory [11, 12]. Recently, numerous studies showed that it widely distributed around non-neuronal cells including endothelial cells, macrophages, dendritic cells (DCs), and keratinocytes [13-15], and activating

$\alpha 7$ nAChR effectively diminished the production of pro-inflammatory cytokines and restored disturbed function of immune cells (Fig. 1)[16, 17]. In $\alpha 7$ nAChR-deficient mice, however, activation of cholinergic system failed to effectively attenuate the excessive inflammation or improve survival rates in critical conditions [14, 18]. Thus, the $\alpha 7$ nAChR might be an effective therapeutic target for inhibiting excessive inflammation and modulating immune homeostasis. Extensive works, focused on exploring effective drugs for activating cholinergic system, have identified that administration of $\alpha 7$ nAChR agonists showed distinct benefits for severe illnesses [19, 20]. However, its specific molecular mechanisms remained unclear. In this review, we attempt to summarize the current molecular mechanisms concerning protective effects of $\alpha 7$ nAChR on acute critical illnesses and provide theoretic basis for seeking novel interventional strategies.

The structure and the function of $\alpha 7$ nicotinic acetylcholine receptor

The groups of nicotinic acetylcholine receptors are ligand-gated ion channels by the combination of at least 17 different subunits that are organized as hetero- or homo-pentamers [21]. The function of each subunit varies with diverse constructions, for instance, β subunits show lower affinity for acetylcholine than α subunits [22]. Therefore, diverse combinations of protein subunits may provide considerable functional variation in the development of receptor subtypes. For example, $\alpha 4\beta 2$ receptors,

another important target for modulating neurological functions, manifest different effects on neurodegenerative diseases with different combinations. They show high affinity for Ach with construction of $(\alpha 4)_2(\beta 2)_3$, while $(\alpha 4)_3(\beta 2)_2$ receptors express low affinity for binding Ach. Other forms of nAChRs, such as $\alpha 3\beta 2$ and $\alpha 3\beta 4$ receptors, are also reported quite different roles in clinical trials [23, 24]. The classic $\alpha 7$ nAChR is composed of five identical $\alpha 7$ subunits which form a central pore with high permeability for calcium [13, 25]. Its structural unicity may contribute to functional uniqueness and rapid response to multiple agonists. These agonists that involve both orthosteric and allosteric modulators can regulate $\alpha 7$ nAChR expression and activation after effective binding [26]. It has been reported that agonists could act as molecular chaperones to induce upregulation and maturation of $\alpha 7$ nAChR, which might not be seen in transcriptional levels. Additionally, agonists induced $\alpha 7$ nAChR activation were identified to be associated with proteins conformation changes characterized by the opening of transmembrane calcium channels [27]. Therefore, the functional states of $\alpha 7$ nAChR changed rapidly and were associated with Ca^{2+} influx: in rest state, the gate was closed and prevented ion flux through the channel, while it transformed into the desensitized states after a transient Ca^{2+} influx upon activation [28-30]. Additionally, the desensitized states of $\alpha 7$ nAChR were reported to happen quickly after binding with agonists, which were associated with different types and function time of agonists,

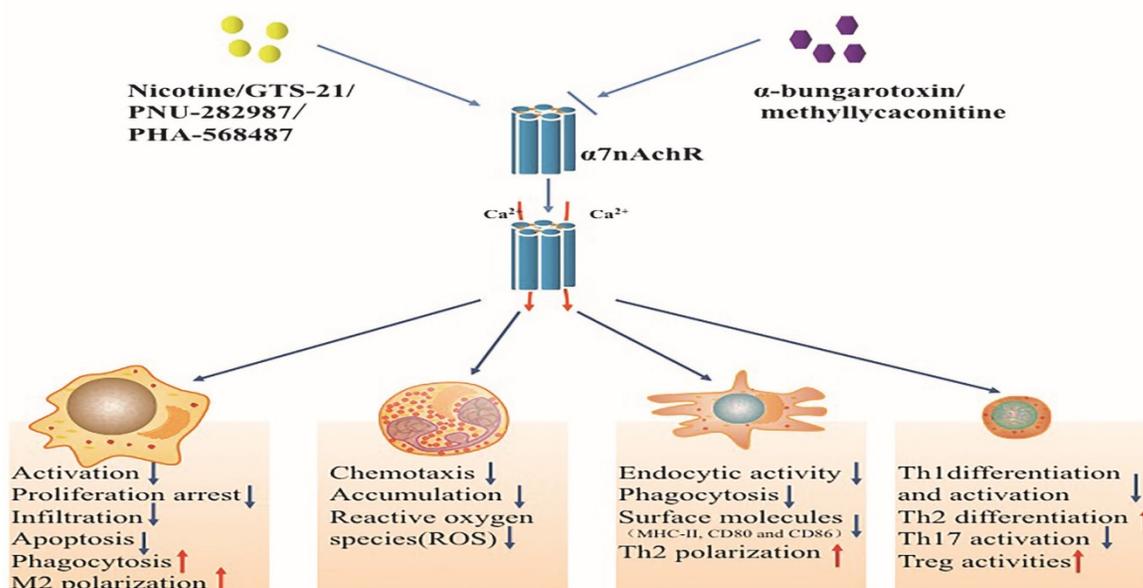


Figure 1 Effects of activated alpha 7 nicotinic acetylcholine receptor ($\alpha 7$ nAChR) on immune cells. The $\alpha 7$ nAChR has been identified to be expressed on multiple immune cells such as macrophages, neutrophils, dendritic cells, and T lymphocytes. The classic $\alpha 7$ nAChR is a homomeric pentamer composed of five putatively identical $\alpha 7$ subunits that form a central pore with high permeability for calcium. Nicotine, GTS-21 and PNU-282987 are typical agonists of $\alpha 7$ nAChR which can activate $\alpha 7$ nAChR followed by transient Ca^{2+} influx. Activated $\alpha 7$ nAChR can affect the function of immune cells while abated by $\alpha 7$ nAChR antagonists including α -bungarotoxin and methyllycaconitine.

mutations of residues and interactions among various linkers [31,32], indicating that the $\alpha 7nAChR$ was initiated early after the onset of inflammation and the functional states of $\alpha 7nAChR$ might be maintained in a certain range by self-regulation.

Stimulation of macrophages with lipopolysaccharide (LPS) or pro-inflammatory cytokines also markedly up-regulated the expression of $\alpha 7nAChR$. Similar results were obtained when endothelium of rat kidney endured ischemia-reperfusion injury [13, 33]. Early administration of nicotine or GTS-21 significantly ameliorated ischemia-reperfusion injury of rat kidney and improved survival of burned mice [33, 34], suggesting early initiation of $\alpha 7nAChR$ in critical settings.

Selective activation of $\alpha 7nAChR$ can benefit many critical conditions (Table 1) [19, 20, 35-38].

Recently, a number of investigations have focused on exploring effective agonists and determining downstream signaling pathway when $\alpha 7nAChR$ was activated. Nuclear translocation of nuclear factor- κB (NF- κB) was a key regulatory factor in modulating activation of immune cells and expression of pro-inflammatory cytokines [39]. Activation of $\alpha 7nAChR$ significantly decreased NF- κB activity, followed by lowered inflammatory response [40]. Further investigations showed that there were various signaling pathways that contributed to inhibiting NF- κB activity after $\alpha 7nAChR$ activated [40, 41]. In the remaining part of this review, we will focus on discussing the association between $\alpha 7nAChR$ activation and development of acute critical illnesses, including underlying mechanisms regarding the protective roles of $\alpha 7nAChR$.

Table 1 The protective effects of $\alpha 7nAChR$ activation on critical illnesses

Types of critical illnesses	Effects of $\alpha 7nAChR$	References
Severe burns/trauma	<ul style="list-style-type: none"> ▲Protecting against cell death ▲Limiting neutrophil priming ▲Preventing gut barrier failure ▲Attenuating local and systemic inflammation ▲Ameliorating organ injury ▲Improving survival rate 	30, 35, 43, 47, 49
Sepsis	<ul style="list-style-type: none"> ▲Enhancing bacterial clearance ▲Down-regulating the expression of pro-inflammatory cytokine ▲Decreasing chemokine production ▲Inhibiting neutrophil migration ▲Preventing systemic inflammation ▲Ameliorating organ injury ▲Improving survival rate 	14, 15, 54, 55, 56
Acute lung injury	<ul style="list-style-type: none"> ▲Alleviating lung edema ▲Inhibiting TNF-α release ▲Lowering gradient of pulmonary alveoli-artery ▲Reducing neutrophil accumulation ▲Decreasing pulmonary apoptosis ▲Maintaining integrity of pulmonary endothelium ▲Improving the outcome 	7, 62, 63, 64, 65
Ischemia-reperfusion injury	<ul style="list-style-type: none"> ▲Reducing serum TNF-α and HMGB1 levels ▲Improving renal function ▲Attenuating tubular cells necrosis ▲Decreasing infarct volume ▲Elevating neurological manifestation ▲Reducing neutrophil infiltration ▲Suppressing superoxide radical generation ▲Reducing oxidative stress ▲Inhibiting systemic inflammatory response 	31, 73, 74, 75, 76
Acute pancreatitis	<ul style="list-style-type: none"> ▲Inhibiting infiltration of pancreatic neutrophils ▲Reducing plasma TNF-α and IL-6 concentrations ▲Decreasing plasma amylase and lipase levels ▲Alleviating severity of experimental pancreatitis 	5, 18

$\alpha 7nAChR$: Nicotinic acetylcholine receptor alpha7; TNF- α : Tumor necrosis factor- α ;
HMGB-1: High mobility group box-1 protein; IL-6: Interleukin-6.

The protective effect of $\alpha 7$ nicotinic acetylcholine receptor activation on critical illnesses

Severe trauma/burns

Severe trauma is characterized by extensive local and systemic inflammation owing to direct mechanical insults, and subsequently induce disturbed immune response and multi-organ failure [42]. Serious burn injury can also bring about septic complications with multiple micro-organisms by damaging mechanical barrier and bacterial contamination [43, 44]. Therefore, restoration of mechanical barrier and inhibition of excessive inflammatory response are major interventional measures.

In experimental models of traumatic brain injury (TBI), the contents of brain $\alpha 7$ nAChR were significantly lowered [45]. However, expressions of $\alpha 7$ nAChR in macrophages and myofibroblasts were up-regulated during the healing stage of skeletal muscle injury, which was also shown in buccal mucosal cells of burn patients [46, 47], suggesting that the expressions of $\alpha 7$ nAChR varied with different pathological conditions and provided by different therapeutic effects. It has been reported that nicotine or GTS-21 utilization could significantly attenuate cognitive dysfunction following experimental TBI and improve burned mice survival by attenuating local and systemic inflammation [33, 48]. And treatment with nicotine could also protect gut barrier through up-regulating the expression of occludin and ZO-1 which were proteins contributing to cellular tight junctions [36].

Septic complications

Sepsis is defined as disturbed responses to multiple infection with its severity driven by the excessive inflammation [49,50]. However, simply antagonization of pro-inflammatory cytokines showed low efficiency in improving septic survival [51], suggesting that the complicated networks of inflammation might be responsible for its high mortality.

Cedillo and colleagues made a comparison of $\alpha 7$ nAChR mRNA levels in peripheral blood monocytes between 33 controls and 33 septic patients, and found that $\alpha 7$ nAChR mRNA levels in septic patients were markedly elevated, which returned to controlled levels after recovery [52]. In addition, $\alpha 7$ nAChR levels were considered to be inversely associated with the magnitude of severity and mortality rate in severe septic patients [52]. Activation of $\alpha 7$ nAChR by GTS-21 significantly improved

survival in septic models, but it failed by antagonizing $\alpha 7$ nAChR or gene knockdown [16, 17]. Further researches showed that stimulation of $\alpha 7$ nAChR alleviated inflammatory response of macrophages [53], and attenuated sepsis-induced acute lung injury by inhibiting chemokines production and neutrophils migration [54, 55]. Additionally, $\alpha 7$ nAChR activation showed benefits for septic conditions by improving bacterial clearance of macrophages [54, 55].

Acute lung injury

ALI is a complication mainly initiated by severe infection, trauma and burns, and it is often followed by acute respiratory failure [56, 57]. ALI is characterized by pulmonary edema, alveolocapillary lesion, and respiratory injury owing to uncontrolled local inflammation [58-60]. Therefore, regulation of the networks of local inflammation may be of great therapeutic value. Pretreatment or delayed administration of nicotine significantly alleviated pathological changes and lung edema in LPS induced ALI models, followed by marked improvement of outcome [61]. GTS-21 also improved lung function in ALI conditions by inhibiting TNF- α production, while these effects were vanished with $\alpha 7$ nAChR antagonist mecamylamine [62]. On the contrary, a deficiency of $\alpha 7$ nAChR showed a two-fold increase of pulmonary permeability and volume of lung water, thus suggesting the therapeutic interest of $\alpha 7$ nAChR in ALI [7, 62]. Further researches have documented that $\alpha 7$ nAChR could benefit ALI models by lowering alveoli-artery gradient, reducing neutrophils accumulation and pulmonary apoptosis [61-63]. Additionally, maintaining the integrity of pulmonary endothelium also appeared to be the effects of $\alpha 7$ nAChR activation [36, 64].

Ischemia-reperfusion injury

Ischemia-reperfusion injury (IRI) is a distinct clinical manifestation and affects many organs such as brain, heart, liver, kidney, and intestine [65-68]. Initiated by ischemic injury, it enhances the production of reactive oxygen species (ROS), and causes calcium overload of intracellular and endothelial cells damage [69]. Subsequent reperfusion stages, however, are characterized by exacerbated tissue injury, uncontrolled systemic inflammation, and multi-organ failure [69]. Accordingly, removal of ROS, alleviation of inflammatory response, and inhibition of cells apoptosis are major interventions [70].

Pretreatment with nicotine or GTS-21 significantly protected tubular cells from necrosis and restored renal function in rats after IRI [34, 71]. Likewise, administration of PHA-543613 decreased

infarct volume and improved cognition in cerebral IRI, while opposite effects were found by giving $\alpha 7nAChR$ blocker α -bungarotoxin [72]. Therefore, the protective roles of $\alpha 7nAChR$ in IRI could be applied to different organs. Activation of $\alpha 7nAChR$ significantly suppressed serum TNF- α and HMGB1 levels during myocardial IRI and resulted in reduction of neutrophils accumulation and attenuation of tubular damage in renal IRI by preventing tubular cells necrosis and myeloperoxidase activity [73]. However, these effects were not significant in delayed treatment, suggesting that $\alpha 7nAChR$ might function in the early stages of IRI conditions [71].

Acute pancreatitis

Acute pancreatitis is a serious ailment with etiologic factors involving gallstones, alcohol, infection and so on [74]. Pancreatic auto-digestion induced by activation of trypsinogen is believed a major factor in the pathogenesis of pancreatitis [75]. Recent evidences have indicated that inflammatory disorder also accounted for its initiation and aggravation [76, 77]. The production of cytokines and chemokines, such as TNF- α , IL-6, monocyte chemotactic protein (MCP)-1, and intracellular adhesion molecule 1 (ICAM-1) were significantly enhanced in acute pancreatitis, and they were independent of trypsinogen activation [77, 78]. Also, infiltration of neutrophils and macrophages were reported to act as key factors in the development of acute pancreatitis with relevant to disease severity. T cells' dysfunction, likewise, accounted for the progression of acute pancreatitis [77]. However, mechanisms underlying the pathogenesis and progress of acute pancreatitis were on elucidated. van Westerloo et al. found that GTS-21 significantly alleviated the severity of experimental pancreatitis by lowering plasma amylase and lipase levels [20]. Less infiltration of neutrophils and low IL-6 concentrations were also noticed in acute pancreatitis preceded by GTS-21 treatment [20]. Furthermore, melanocortin peptides exerted protective effects in acute pancreatitis as shown by blunted plasma amylase and lipase activity, decreased TNF- α and IL-6 levels, and reduced neutrophils accumulation, which were also associated with activating cholinergic system [5].

Molecular mechanisms of $\alpha 7nAChR$ action

$\alpha 7nAChR$ and inflammation

Interactions between $\alpha 7nAChR$ and pro-inflammatory cytokines involve numerous molecular pathways (Fig. 2). Selective activation of

$\alpha 7nAChR$ has been identified to down-regulate pro-inflammatory cytokines expressions [1]. Additionally, pro-inflammatory cytokines, HMGB1 as an example, could markedly up-regulate the expression of $\alpha 7nAChR$ [13], suggesting that there might be bilateral interactions between $\alpha 7nAChR$ and inflammatory mediators. Clinical studies have also reported negative correlation between concentrations of $\alpha 7nAChR$ agonists and pro-inflammatory mediators' levels [79].

Wang et al. found that activation of $\alpha 7nAChR$ could significantly block NF- κ B signaling [32]. Various mechanisms were involved in interfering with NF- κ B activation. For instance, $\alpha 7nAChR$ -Janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3)-NF- κ B cascades were the most studied, which significantly suppressed activity of NF- κ B after $\alpha 7nAChR$ activation [41, 80]. Blocking JAK2 with AG490, a selective antagonist of JAK2 phosphorylation, dampened the inflammatory regulating effects of $\alpha 7nAChR$ agonists, same effects were exhibited by inhibiting STAT3 phosphorylation [80], indicating that JAK2-STAT3 might function as pivotal bridge between $\alpha 7nAChR$ and NF- κ B regulation. I κ B, which was recognized as a key regulator of NF- κ B, prevented the NF- κ B activation in resting state [81]. It dissociated from NF- κ B after being activated by pathogen associated molecular patterns (PAMPs), and subsequently induced NF- κ B translocation [82]. Administration of nicotine to monocytes resulted in suppressed ubiquitination of I κ B and further inhibited activity of NF- κ B by depressing I κ B kinases (IKK) activity [40]. Other associated mechanisms included Myeloid differentiation factor 88 (MyD88) that was a key intra-cellular reactive protein for Toll-like receptor (TLR) and acted as an universal adapter for TLRs, including TLR2, TLR4, TLR5, TLR7, and TLR9. Studies have shown that MyD88 could activate IKK and further induce NF- κ B translocation upon activation [83]. MyD88 expression was increased when exposed to LPS, while nicotine decreased the expression of MyD88, and subsequently interfered NF- κ B transcription [84]. Similar effects were demonstrated in HBE16 cells transfected with MyD88 siRNA, suggesting that MyD88 was a potential target for $\alpha 7nAChR$ with regulating cytokines profiles [84].

Other than controlling acute inflammation by inhibiting NF- κ B activity, a number of negative regulatory factors are also recognized concerning with $\alpha 7nAChR$ downstream pathways. Interleukin-1 receptor-associated kinase M (IRAK-M) belongs to the IRAK family but with kinase activity absent, which is widely expressed in monocytes and macrophages [85]. IRAK-M expression is a negative regulator of

TLR activity through preventing the dissociation of active IRAKs (IRAK-1 and IRAK-4) from MyD88, and it can interfere active TLRs downstream signalings by binding with TNF receptor-associated factor 6 (TRAF6)[86]. Maldifassi and colleagues have proved that nicotine enhanced IRAK-M expression at both gene and protein levels in macrophages by activating JAK2/STAT3 or PI3K/STAT3 cascade, resulted in decreased production of TNF- α [87]. Silencing IRAK-M gene significantly reversed the anti-inflammatory effects of nicotine [87]. Other signals involved in the anti-inflammatory mechanisms of α 7nAChR were also present effectively. Taking PI3K-Akt pathway for example, activity of PI3K-Akt was significantly enhanced after treatment with donepezil that could activate α 7nAChR, followed by alleviated

neuro-inflammation [88]. In addition, activation of α 7nAChR distinctly diminished TLR4 expression via increasing PI3K/Akt activation, thereby leading to reduction of inflammatory cytokines and improvement of sepsis survival [89]. α 7nAChR was also identified expressed in mitochondria, and it could regulate cytokines production and cellular apoptosis after being activated by intracellular acetylcholine (Ach) [90-92]. Further studies indicated that activated mitochondrial α 7nAChR significantly suppressed mitochondrial DNA release and activation of NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome, as a result, expressions of IL-1 β and HMGB1 were suppressed [92]. Therefore, the anti-inflammatory mechanisms of α 7nAChR involve various locations and multiple levels.

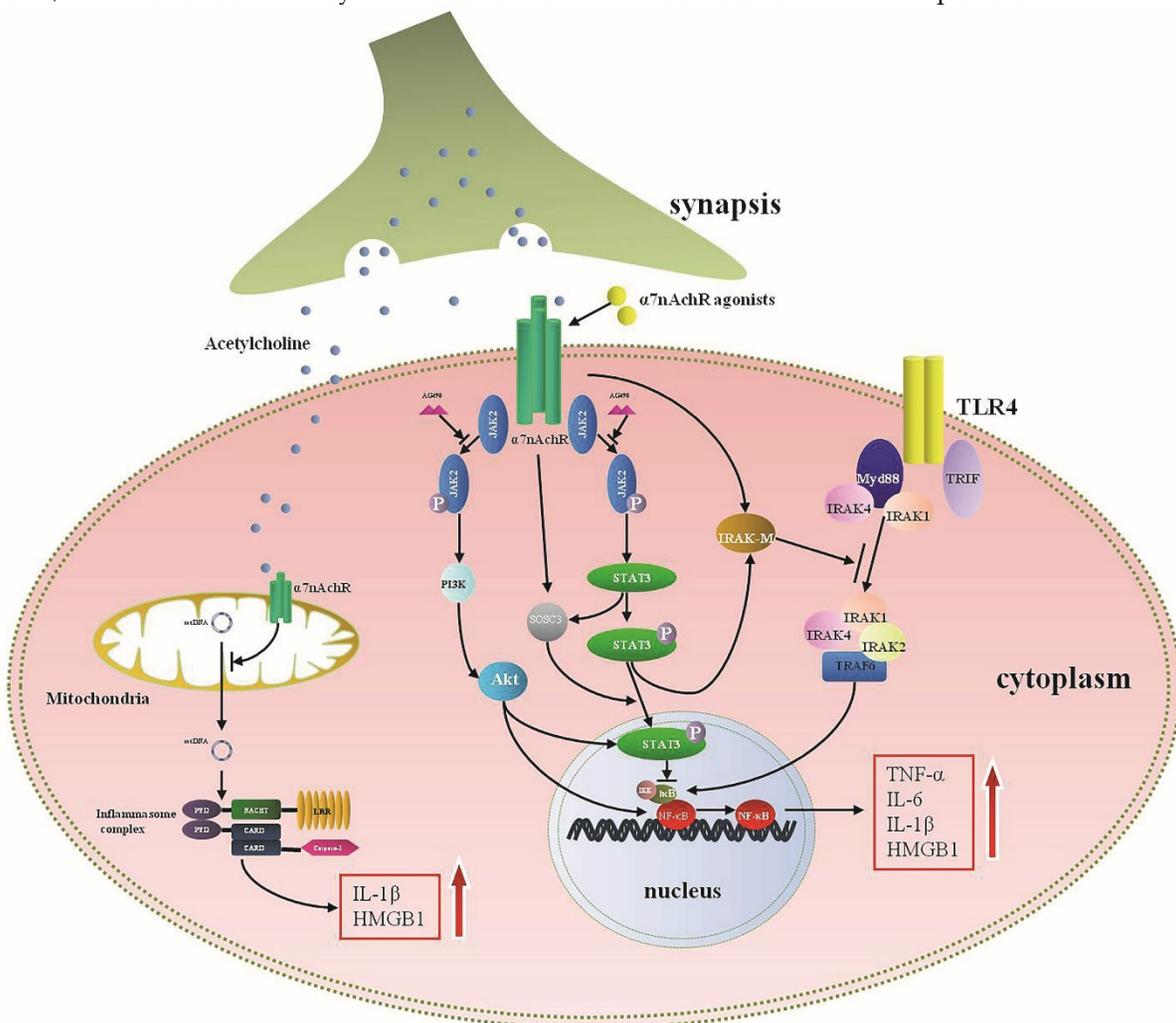


Figure 2 The intracellular signaling pathways of α 7nAChR in anti-inflammatory response. Acetylcholine (Ach), released from the terminal of synapsis, is a primary endogenous agonist of α 7nAChR and exerts anti-inflammatory effect by activating α 7nAChR. Activated cytomembrane α 7nAChR inhibits the production of pro-inflammatory cytokines mainly through blocking nuclear factor- κ B (NF- κ B) activity, which may involve multiple intracellular cascades. Janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3) pathway represent a typical anti-inflammatory cascade for α 7nAChR which can be blocked by AG490, a selective antagonist of JAK2. Phosphorylated STAT3 suppresses the dissociation between I κ B and NF- κ B, and subsequently inhibits the activation of NF- κ B. Activated STAT3 also up-regulates the expression of suppressor of cytokine signaling 3 (SOCS3) and interleukin-1 receptor-associated kinase M (IRAK-M), both are essential for anti-inflammatory reaction. IRAK-M can negatively regulate Toll-like receptor (TLR) activity by preventing the dissociation of active IRAKs (IRAK-1 and IRAK-4) from MyD88 and subsequently bonding with TNF receptor-associated factor 6 (TRAF6), thus it can interfere active TLR downstream signalings and inhibit the expression of pro-inflammatory cytokines. Synergistically, PI3K-Akt pathway can attenuate the pro-inflammatory cytokine production when activated by α 7nAChR agonists. Mitochondria are reported to express α 7nAChR. Intra-cellular acetylcholine can inhibit production of IL-1 β and HMGB1 by suppressing release of mitochondrial DNA and inhibiting activation of NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome.

$\alpha 7$ nAChR and immune function

Monocytes and macrophages

Macrophages are believed to be the main source of inflammatory mediators during the courses of acute inflammation [93]. In the initial phases of inflammation, macrophages are activated and migrate into target lesions to remove microbes and associated metabolite and apoptotic cells. This process involves the synergistic effects of endothelials and multiple signal molecules, including MCP-1, interferon (IFN)- γ , GM-colony stimulating factor (CSF), and G-CSF [94]. Activated macrophages can also secrete macrophage inflammatory protein-1 α (MIP-1 α), MCP-1, and IL-8 to induce more macrophages recruitment and activation [94]. Additionally, factors that targeting polarization of M1 or M2 can significantly alter the outcome of diseases as well [95]. In critical illness, however, the structure as well as the function of macrophages becomes abnormal, as manifested by over-activation, impaired phagocytosis and apoptosis and attenuated bacterial eradication [96-98].

GTS-21 has been reported to alleviate proliferation arrest of macrophages induced by LPS [33]. Ravikumar and colleagues have also noted that enhanced bacteria eradication was seen in hyperoxia implicated macrophages after treated by GTS-21, which were related to improved macrophages phagocytosis [55]. Hence, $\alpha 7$ nAChR acted as an effective target for alleviating tissue damage and improving outcome partly through regulating immune function of macrophages in critical diseases. Other important pro-survival and anti-inflammatory effects were that activated $\alpha 7$ nAChR could abate macrophages apoptosis and induce M2 subtypes differentiation [99].

Neutrophils

Neutrophils are important effectors of innate immune response, and mobilized early in acute inflammation [100]. Nonetheless, studies have uncovered multiple dysfunctions of neutrophils, mainly characterized by impaired chemotactic activity and delayed apoptosis, resulted in inadequate neutrophil migration [101, 102]. Neutrophils dysfunctions contributed to increased incidence of nosocomial infections, and excess accumulation of neutrophils was noted as major damage to tissues in critical illnesses [101, 103]. Thus, properly regulating the chemotaxis and apoptotic activities of neutrophils might be beneficial for lessening tissue injury.

GTS-21 effectively modulated accumulation and activation of polymorphonuclear neutrophils (PMNs), $\alpha 7$ nAChR^{-/-} mice, however, showed uncontrolled

PMN infiltration and excessive production of TNF- α and CXCL-1 in comparison with wild-type mice after being challenged with immune complex [104]. Though a series of investigations showed that pro-inflammatory cytokines, TNF- α as an example, rendered neutrophils to accumulate in inflammatory sites, but simply lowering TNF- α production did not significantly inhibited neutrophils accumulation [105]. In severe infection or trauma conditions, some major chemokines, such as CXCL-1 and IL-8, which were crucial for neutrophils migration, showed significantly enhanced production [106]. Nicotine was confirmed to inhibit neutrophils infiltration through reducing CXCL-1 and IL-8 levels [107, 108]. In addition, studies showed that prevention of tissue injury by stimulating $\alpha 7$ nAChR might be partly due to a decreased generation of neutrophils ROS by blunting Fc γ Rs or C5aRs [104]. Therefore, protective mechanisms of $\alpha 7$ nAChR on neutrophils mainly aimed at migrating affected neutrophils to kill invading pathogens and alleviating tissue injury. Nevertheless, $\alpha 7$ nAChR activation has not been evidenced in regulating delayed apoptosis of neutrophils under severe conditions [109].

Dendritic cells

DCs are specific with antigen presenting and known to bridge the innate and adaptive immune systems by inducing the Th cells polarization [110, 111]. Immature DCs show low competence for stimulating Th cells but with advantages of capturing and processing antigens. However, they express major histocompatibility complex (MHC)-I, MHC-II and other costimulatory molecules, including CD80, CD86, and CD40 when they become mature [111]. Therefore, dysfunction of DCs will result in altered immune response. In critical illness, such as severe sepsis, DCs suffered from decreased number due to elevated apoptotic activities and immune dysfunctions which involved reduced expressions of surface molecules, decreased cytokines secretion and inhibited competency for stimulating T cells [112, 113].

Studies have shown that immature DCs developed with lowered endocytic and phagocytic activities after $\alpha 7$ nAChR activation. Mature DCs, likewise, manifested reduced expressions of surface molecules and decreased production of IL-12 under nicotine exposure [114], indicating that the effects of $\alpha 7$ nAChR on DCs focused on attenuating excessive inflammation and maintaining DCs functional homeostasis, which varied with cells situations. Further studies revealed that these effects were associated with up-regulation of peroxisome proliferator-activated receptors- γ (PPAR- γ) [115].

Additionally, secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor-related peptide (SLURP)-1, which was an endogenous $\alpha 7$ nAChR allosteric ligand and expressed in DCs, enhanced $\alpha 7$ nAChR action by increasing Ach levels [116]. However, few studies showed the association between $\alpha 7$ nAChR action and apoptosis of DCs.

T lymphocytes

T cells can be divided into three types, namely Th cells, cytotoxic T cells (CTL), and regulatory T cells (Treg) based on different immune functions [117]. Th cells are the most important component of adaptive immune system, and they are divided into Th1, Th2, Th17 subgroups under different cytokines stimuli [118]. Therefore, dysfunctions of Th cells are closely related to the outcome of severe illness.

The interaction between $\alpha 7$ nAChR and CD4⁺ T cells was considered to be of great importance in immune response. CD4⁺ T cells were essential for the CAP, as evidenced by reestablishment of anti-inflammatory potential of $\alpha 7$ nAChR agonists that failed to suppress TNF- α production in nude mice but succeeded with CD4⁺CD25⁻ lymphocytes transfer [119]. Therefore, effective modulation of T cells' function might enhance the protective effects of $\alpha 7$ nAChR [119]. Ach was also synthesized and released by T cells in autocrine or paracrine fashion, indicating a possible self-regulatory mechanism of T cells in maintaining functional homeostasis [120]. The same role was seen with SLURP-1 that could be provoked by T cells to activating $\alpha 7$ nAChR [120]. Accumulating evidences showed that $\alpha 7$ nAChR activation markedly inhibited Th1 differentiation and activation but augmented Th2 response [121]. Further researches identified that these effects resulted from altered expressions of surface molecules on DCs and suppressed activities of T cells' NF- κ B after $\alpha 7$ nAChR stimulation [32, 114]. In $\alpha 7$ nAChR^{-/-} animal models, however, T cells were more prone to differentiate into Th1 lineages [18]. Other action targets, which were accounted for the modulation of $\alpha 7$ nAChR in T cells, involved Th1 cell-specific T-box transcription factor (TBX21). GTS-21 significantly reduced the expression of TBX21 that was important in facilitating Th1 proliferation [122]. Takahashi and colleagues found that nicotine administration was able to down-regulate the expressions of adhesive molecules on monocytes and associated ligands of T cells, followed by suppressed cells interaction [123]. Enhanced prostaglandin E₂ production was also responsible for the suppression of mixed lymphocyte reaction upon nicotine administration while vanished effects were shown by reducing protein kinase A

(PKA) and cyclooxygenase (COX)-2 activities [123]. Th17 cells functioned as pro-inflammatory immune cells by releasing cytokines including IL-17A, IL-17F, and IL-22 [124]. Th17 cells also accounted for host immune response to invading pathogens and inflammation, but excessive production of cytokines, the IL-17 as an example, could definitely harm the host [125]. Nicotine was reported to decrease Th17 cytokines' levels and suppress Th17 activation in a dose-independent pattern [121].

Tregs are characterized by immunosuppressive properties and play important roles in immune homeostasis [126-128]. Expressions of $\alpha 7$ nAChR were shown on CD4⁺CD25⁺ Tregs [129], and $\alpha 7$ nAChR activation augmented the suppressive effects of CD4⁺CD25⁺ Tregs by up-regulating expressions of cytotoxic T-lymphocyte-associated antigen (CTLA)-4 and forkhead/winged helix transcription factor p3 (Foxp3), and decreasing IL-2 levels [129], indicating that $\alpha 7$ nAChR cascades might evoke multiple anti-inflammatory mechanisms of T cells to maintain immune homeostasis.

Conclusions

The $\alpha 7$ nAChR has been identified to be beneficial for serious conditions, as mainly manifested by attenuation of excessive inflammation and improvement in survival rates of animal models. These effects were closely related to its distinct structure characterized by high permeability for calcium and affinity to acetylcholine, which enabled $\alpha 7$ nAChR respond quickly in the process of inflammation. $\alpha 7$ nAChR activation exhibited extensive anti-inflammatory reactions, including lowered pro-inflammatory cytokines levels, decreased expressions of chemokines as well as adhesion molecules, and altered differentiation and activation of immune cells. All these effects were believed to be beneficial for attenuating excessive inflammation, but might be considered to harm the immune response to microbial infection in the early stages. However, little researches showed detrimental effects of $\alpha 7$ nAChR agonists with pre-treatment or early administration, which might be due to self-regulation of $\alpha 7$ nAChR as an ion channel under physiological conditions, which maintained hemostasis by self-regulation [130]. Additionally, the $\alpha 7$ nAChR was reported to become desensitization in the early stages of critical conditions, which might also account for uncontrolled inflammation, and reversing its desensitization would be of great importance to attenuate excessive inflammation [31]. Furthermore, multiple signaling pathways were responsible for the anti-inflammatory mechanisms of $\alpha 7$ nAChR cascades, represented by

JAK2-STAT3-NF- κ B axis that interacted with other pathways to enhance its protective effects synergistically. With more and more protective evidences of α 7nAChR have been shown in animal models with severe conditions. A few studies have already focused on exploring effective drugs concerning α 7nAChR activation in clinical settings, which were based on some successful results by improving cognition in neurodegenerative diseases [131]. Taking GTS-21 for an example, it significantly inhibited LPS induced cytokines expression in human trials and modulated innate immune responses [79]. Additionally, new agonists of α 7nAChR have also been evaluated in clinical trials, which might be promising in improving neurological dysfunction [132]. Thus, it was of great importance to have a good understanding of protective mechanisms of α 7nAChR relevant to clinical settings.

Abbreviations

α 7nAChR: alpha 7 nicotinic acetylcholine receptor; CAP: cholinergic anti-inflammatory pathway;ALI: acute lung injury; TNF- α : tumor necrosis factor- α ;IL-1 β : interleukin-1 β ; HMGB1: high mobility group box-1 protein; DCs: dendritic cells; LPS: lipopolysaccharide; NF-Kb: nuclear factor- κ B; TBI: traumatic brain injury; HO-1: heme oxygenase; IRI: Ischemia-reperfusion injury; ROS: reactive oxygen species; MCP-1: monocyte chemotactic protein-1; ICAM-1: intracellular adhesion molecule 1; JAK2: Janus kinase 2; STAT3: signal transducer and activator of transcription 3; PAMPs: pathogen associated molecular patterns; IKK: I κ B kinases; MyD88: Myeloid differentiation factor 88; TLR: Toll-like receptor; IRAK-M: Interleukin-1 receptor-associated kinase M;TRAF6: TNF receptor-associated factor 6; IFN- γ : interferon (IFN)- γ ; GM-CSF: GM-colony stimulating factor; MIP-1 α : macrophage inflammatory protein-1 α ; PMNs: polymorphonuclear neutrophils; MHC: major histocompatibility complex; PPAR- γ : peroxisome proliferator-activated receptors- γ ; SLURP-1: secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor-related peptide -1; CTL: cytotoxic T cells; Treg: regulatory T cells; TBX21: Th1 cell-specific T-box transcription factor; PKA: protein kinase A; COX-2: cyclooxygenase-2; TGF- β : transforming growth factor; CTLA-4: cytotoxic T-lymphocyte-associated antigen-4; Foxp3: forkhead/winged helix transcription factor p3.

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Competing Interests

The authors have declared that no competing interest exists.

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