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REVIEW

Nicotine, an anti-inflammation molecule

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Nicotine, as one of the most important components of cigarette smoke and the key contributor of initiating and maintaining tobacco dependence, has anti-inflammatory effect in the cells of both nervous system and immune system. Among the different types of subunits of nicotinic acetylcholine receptors (nAChR), α7 nAChR is related to the immune response. Nicotine exerts anti-inflammatory effect through cholinergic anti-inflammatory pathway by binding to α7 nAChR. In this review, we summarized the molecular mechanisms of the anti-inflammatory effect of nicotine via binding to the α7 nAChR. We also reviewed the current findings of the nicotinic anti-inflammatory effect in various diseases and disorders. Especially, we focused on the nicotinic anti-inflammatory effect on influenza A virus infection.

Keywords: nicotine; anti-inflammatory effect; influenza A virus; cigarette smoke

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Introduction

Cigarette smoking has been regarded as the leading preventable cause for death and disability worldwide. The global epidemic of cigarette smoking leads to approximately 6 million deaths every year and secondhand cigarette smoke exposure accounts for more than 10% of these deaths [1]. The majority of cigarette smoking related deaths are due to cardiovascular diseases, such as stroke and heart failure, peripheral vascular disease; respiratory diseases, such as chronic obstructive pulmonary disease; and cancer, such as bladder cancer and lung cancer. Cigarette smoking might also contribute to wrinkles and premature aging, gum disease, impaired wound healing, diabetes, enhanced risk of viral and bacterial infection as well as increased risk of asthma. Furthermore, cigarette smoking is associated with the infertility in both women and men, as well as an increased risk of sexually transmitted diseases [2]. However, there are increasing evidence indicated that smokers may have lower incidence to suffer from certain diseases [3]. Clinical and epidemiological studies demonstrate that cigarette smoking might decrease the occurrence and progression of ulcerative colitis [4] and reduce the risk of sarcoidosis [5, 6], Parkinson's disease [7] and Sjogren's syndrome [8].

Mainstream cigarette smoking can generate about 5,600 chemicals, among which, nicotine is one of the most important components and responsible for initiating and maintaining tobacco dependence [9, 10]. Nicotine exerts its anti-inflammatory effect through cholinergic anti-inflammatory pathways by binding to and activating α7 nAChR [11, 12].

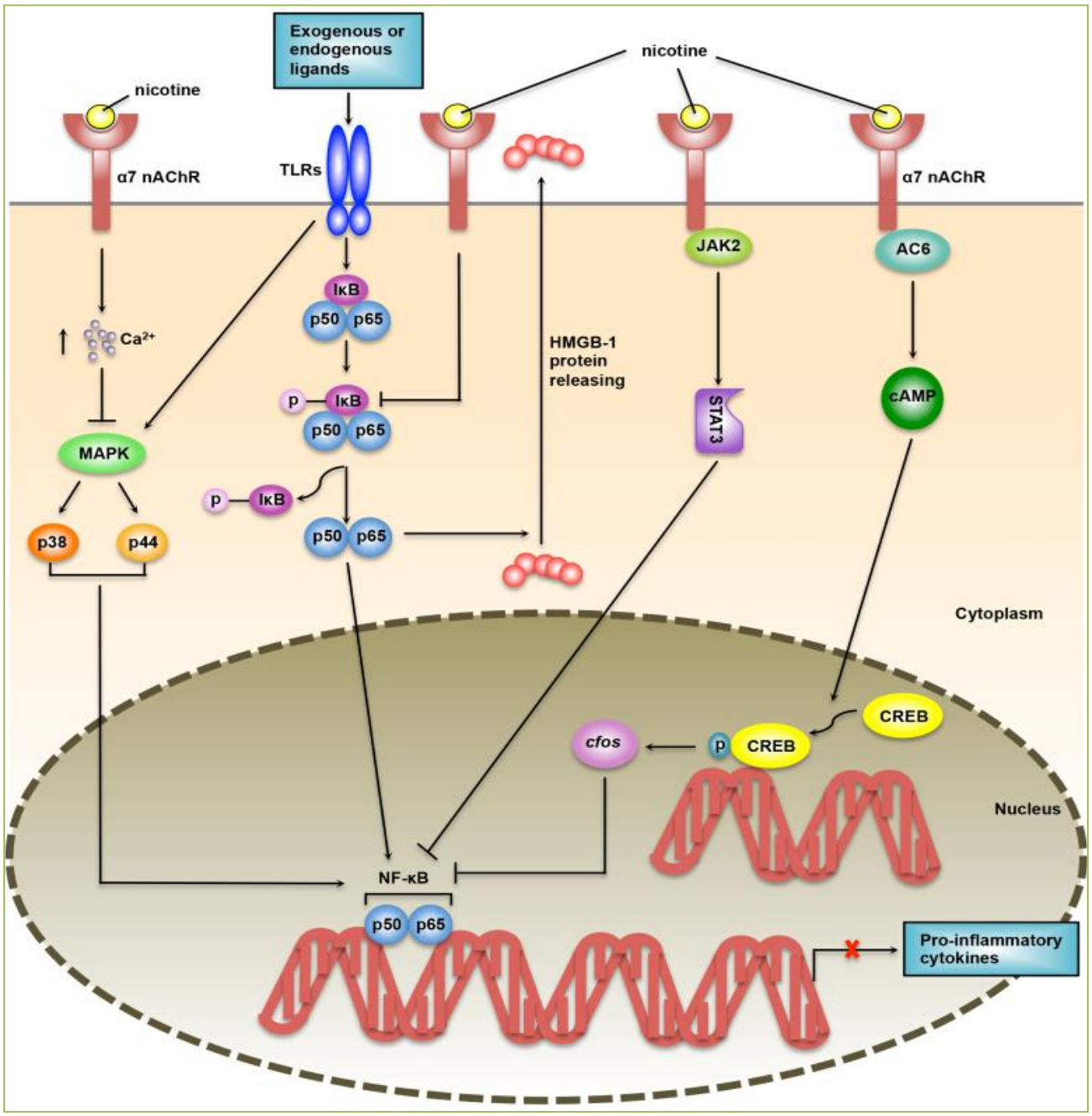

Figure 1. Simplified molecular mechanism of the cholinergic anti-inflammatory pathway mediated anti-inflammatory effect via α7 nAChR. In the cholinergic anti-inflammatory pathway, nicotine binds to the α7 nAChR and finally reduces the expression of proinflammatory cytokines. TLR, toll like receptor; α7 nAChR, α7 nicotinic acetylcholine receptor; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; IκB, inhibitor of κB; HMGB1, high-mobility group box chromosomal protein 1; JAK2, janus kinase 2; STAT3, signal transducer and activator of transcription 3; AC6, adenylate cyclase.

Our recent murine study reported the novel findings that the 2009 pandemic H1N1 (pdmH1N1) and avian H9N2 (H9N2/G1) influenza A virus infection induced disease severity was ameliorated by prior cigarette smoke exposure [13]. Such favorable consequent was attributed to the immunosuppressive effect of cigarette smoke expo- sure that decreased the excessive inflammatory response caused by influenza A virus infection. The anti-inflammatory effect of nicotine might be responsible for this beneficial outcome [13]. The novel findings aroused our interest and urged us to improve our systematic understanding of the anti-inflammatory effect of nicotine. This article offers an updated review of our present under- standing of the molecular mechanisms of the nicotine anti-inflammatory effect and highlights the relationship between the anti-inflammatory effect of nicotine and influenza A virus

infection.

Characteristics of nicotine

Nicotine, as one of the key components of cigarette smoking, accounts for approximately 1 to 2 percent of the total chemicals and is responsible for initiating and maintaining the dependence of tobacco [14]. Nicotine hydrogen tartrate salt with an anhydrous MW of 462 and free base with MW of 162 are the two forms that the commercially available nicotine exists. In tobacco, the predominant nicotine active form in pharmacology is (*S*)-nicotine rather than the (*R*)-nicotine [15]. Ionized nicotine and non-ionized nicotine are 69% and 31% respectively in the pH7.4 of bloodstream, and less than 5% of nicotine binds to plasma proteins. Compared with adipose tissue that displays the lowest affinity, tissues of liver, lungs, kidney and spleen show the highest affinity for nicotine. The nicotine is rapidly absorbed by the lungs after inhaling the cigarette smoke, and reaches to a rather high blood concentration that leaves the heart, and then could reach the brain within 8-10 seconds. The average nicotine eradication half-life in plasma is different in different animals. It is about 2 hours in humans and nonhuman primates, 45 minutes in rat and approximately 6-7 minutes in the mouse [15].

There are six primary nicotine metabolites which are mainly metabolized by liver in mammalian species, including cotinine, cotinine glucuronide, nicotine glucuronide, nicotine-*N*'-oxide, *trans*-3'-hydroxycotinine glucuronide and *trans*-3'-hydroxycotinine. Cotinine is the governing metabolite and accounts for about 70-80 percent. Several minor metabolites could also be found. It is liver enzyme CYP2A6 that metabolizes nicotine to cotinine and cotinine to 3'-hydroxycotinine [15]. Animal species of monkeys, dogs, rabbits and mice metabolize nicotine similarly as humans, while guinea pigs and rats do not [15]. In humans, the metabolism of nicotine might be affected by the sex, age, disease states and race. There is one study demonstrated that women metabolized both nicotine and cotinine faster than men [16]. Adult smokers clear total nicotine faster than elderly people but much more slowly than younger smokers [17]. Patients with chronic kidney disease have decreased renal clearance of both nicotine and cotinine. Furthermore, stress, which is a critical environmental factor, is also related to the metabolism of nicotine, especially in animal model [15]. It was also reported that Caucasians cleared both nicotine and cotinine much more rapidly than Asians and African Americans do [15].

Nicotinic acetylcholine receptors

Acetylcholine receptors (AChRs) have two main subtypes: the ionotropic nicotinic receptors and the metabotropic muscarinic receptors [18]. In 1905, nicotine was first reported to be one of the nAChRs receptive ligands [19]. Nicotine exerts its function through directly binding to nAChRs, which are located on the cytoplasmic membrane. The nAChRs are ionic channels and made up of five homologous or identical subunits that form an ionic pore and the ligand-binding site [20]. The nAChRs show widespread tissue distribution and might be found in peripheral and central nervous system, and other non-neuronal or non-muscle cells, such as macrophages, keratinocytes, epithelial cells and so on [18].

So far, total 17 different types of nAChRs subunits have been characterized in humans; they are α1-α10, β1-β4, γ, δ and ε [20]. Different subunits show different affinities for nicotine. It was reported that receptors consisting of the α4 and β2 subunits usually displayed higher nicotine affinities [21].

Different nAChRs subunits are usually involved in different activities. Subunit of α3 functions in autoimmune autonomic gangliopathy and palmoplantar pustulosis; α7 is related to the immune responses; and β2 plays a vital role in addiction, pain, epilepsy, heart disease, schizophrenia, Parkinson's disease and Alzheimer's disease [22]. There was a report showed that subunits of α1, β1 and δ along with either ε (adult) or γ (fetal) might form heteropentameric nicotinic receptors to regulate muscle contraction [11]. It has also been reported that multiple subunits of nAChRs, such as α7, β2 and β4, could be identified on different types of bone marrow derived immune cells, particularly the macrophages [11, 23], implying that they might play a role in the immune responses.

Cholinergic anti-inflammatory pathway

Appropriate inflammation plays an important and beneficial role in many human diseases but excessive inflammation might lead to morbidity and mortality. Fortunately, human bodies develop a series of endogenous and extensively conserved mechanisms that could regulate and control the immune responses to prevent the hyper-reaction of inflammation. For example, the release of tumor necrosis factor (TNF) in macrophages could be significantly and rapidly inhibited by the vagus nerve of the nervous system, and then the systemic inflammatory responses were alleviated. Such physiological mechanism is called "cholinergic anti-inflammatory pathway" [24].

Until now, more and more evidence have demon- strated that the cholinergic anti-inflammatory pathway plays an important role in modulating the immune cells functions in multiple inflammatory diseases [25]. The physiological interaction between α7 nAChR and its agonists directly activates the cholinergic anti-inflammatory pathway. The agonists of α7 nAChR include neurotransmitter acetylcholine (ACh), which is produced by lymphocytes or

the parasympathetic nervous system [26]; choline; nicotine and experimental therapeutic GTS-21 [27]. The α7 nAChR has broad distribution on the cells of both nervous system and immune system, including monocytes, macrophages, DCs, B cells, T cells [27] as well as airway respiratory epithelial cells [28]. The molecular mechanism of the cholinergic anti-inflammatory pathway mediated anti-inflammatory effect via α7 nAChR is shown in Figure 1.

In response to multiple stimulations, such as exogenous or endogenous ligands of TLR2, TLR3, TLR4, TLR7 and TLR9, α7 nAChR and its agonists could exert their anti-inflammatory effect through the cholinergic anti-inflammatory pathway to suppress the production of cytokines in macrophages and monocytes [29].

In monocytes or macrophages, the physiological interaction between α7 nAChR and its agonists, such as nicotine, might suppress the phosphorylation of IκB, which is the NF-κB inhibitor [30]; or stimulate the physical interaction between α7 nAChR and adenylate cyclase 6 (AC6) to increase the intracellular cAMP level, which could activate the phosphorylation of CREB and then enhance the production of *cfos* (one member of the immediate early gene family of transcription factors and could negatively regulates NF-κB signaling pathway); or stimulate the recruit of janus kinase 2 (JAK2) to form a heterodimeric complex, and then activate signal activator and transducer of transcription 3 (STAT3), which negatively regulates NF-κB signaling pathway. Together, all of these signaling cascades lead to the suppression of proinflammatory cytokines up-regulation by NF-κB [12, 27]. High-mobility group box chromosomal protein 1 (HM GB1) was found not only a transcription and growth factor, but also could act as a proinflammatory cytokine that involved in stimulating the production of IL-6, IL-1β and TNF, and mediating the inflammation in many diseases, including sepsis [31-36]. The HMGB1 protein releasing was controlled by NF-κB, and the activation of α7 nAChR by nicotine was found to inhibit HMGB1 production by suppressing the activity of NF-κB [20, 36]. In microglial cells, the activation of α7 nAChR can transiently up-regulate the intracellular calcium levels and then reduce the phosphorylation of the MAPKs p38 and p44 [37, 38]. Similar mechanisms were also observed in T cells [39]. These mechanisms finally could induce the decreased expression of proinflammatory cytokines [40].

Anti-inflammatory effect of nicotine

Nicotine, as a nicotinic cholinergic agonist, could bind to α7 nAChR and then activate the cholinergic anti-inflammatory pathway. In 2003, one study firstly demonstrated that nAChR α7 subunit played a key role in suppressing cytokine production in response to nicotine stimulation. After treating the wild type mice and α7 nAChR knockout mice with bacterial endotoxin of lipopolysaccharide (LPS), the serum TNF-α level was significantly higher in α7 nAChR knockout mice than that in wild type mice. Nicotine or ACh stimulation had no effect on TNF-α expression in LPS treated peritoneal macrophages that were derived from α7 nAChR knockout mice [11]. Animal study also indicated that nicotine treatment could down-regulate the production of IL-1, IL-6, TNF-α, MIP-1α/CCL3, MIP-2/CXCL2 and eotaxin /CCL11; the recruitment of leukocytes and the edema in BAL of mice after LPS stimulation [41]. For the effect of nicotine during virus or virus-like infection, it was reported that poly (I:C)-induced inflammatory response in mouse macrophages could be suppressed by nicotine significantly. Specifically, nicotine could attenuate the mRNA expression of IL-6, IL-1β and TNF-α in poly (I:C) stimulated peritoneal macrophages; and suppress the production of IL-6 and TNF-α from poly (I:C) stimulated macrophages [42]. Nicotine pretreatment might prevent the mice from renal dysfunction during kidney ischemia/reperfusion injury by binding to the α7 nAChR, and then preventing neutrophil recruitment, decreasing tubular damage as well as reducing the production of TNF-α, KC/CXCL1 and proinflammatory HMGB1 protein in a dose-dependent manner [43]. Activation of the cholinergic anti-inflammatory pathway through α7 nAChR by nicotine might also attenuate the neuro- inflammation via inhibiting Th1 and Th17 responses and decreasing T cell proliferation [44]. It was also demonstrated that in the mouse experimental autoimmune encephalomyelitis (EAE) model, nicotine treatment could significantly down-regulate and delay inflammatory and autoimmune responses in central nervous system [45]. Furthermore, nicotine stimulation might significantly protect the mice from obesity and ulcerative colitis by activating the cholinergic anti- inflammatory system via α7 nAChR [46].

Nicotine stimulation also shows protective anti-inflammatory effect in the clinical human study. In normal subjects, LPS-mediated systemic inflammatory responses could be attenuated by transcutaneous nicotine administration via decreasing cardiovascular responses and temperature, but increasing levels of circulating IL-10 and cortisol [47]. Treating the rheumatoid arthritis patients derived fibroblast-like synoviocytes cells with nicotine could significantly inhibit TNF-α-induced secretion of IL-8 and IL-6 by suppressing the TNF-α- induced NF-κB nuclear translocation [48]. Moreover, both human and animal clinical studies reported that nicotine and nAChR drugs indicated promising therapeutic agents for reducing the incidence of Parkinson's disease [49].

The anti-inflammatory effect of nicotine on influenza A virus infection

Influenza viruses are member of the family of

Orthomyxoviridae and are negative-sense, single stranded RNA viruses. Until now, three types of influenza viruses, influenza A, B and C have been identified based on the antigenicity of matrix protein and nucleoprotein [50, 51]. It is reported that influenza A of H1N1 and H3N2 and influenza B viruses are responsible for the recurrent annual seasonal epidemics [52-54]. However, when taking the annual epidemics and the sporadic outbreak of pandemics into account, the influence of influenza A viruses is much more severe than that of influenza B viruses [52]. According to genetic and serological differences of hemagglutinin (HA) and neuraminidase (NA), which are the two surface glycoproteins, influenza A viruses can be divided into a large number of unique subtypes, with different combination of 17 HA and 9 NA [54, 55]. So far, influenza A viruses have caused significant morbidity and mortality in both human and animals [53, 56].

Inflammation has double-edged sword characteristics; appropriate inflammation is critical in modulating the inflammatory responses, eradicating pathogens and subsequent repairing of the injured tissues， whereas excessive inflammatory response would be extremely harmful. Both innate and adaptive immune responses could be triggered shortly after influenza A virus infection. The influenza A virus infection induced antiviral signaling cascades lead to the production of proinflammatory cytokines, chemokines and IFNs to activate the antiviral responses [54]. Then, how about the anti-inflammatory effect of nicotine on influenza A virus infection? In 1998, one study reported that 2 weeks nicotine treated mice had longer survival than control mice after a lethal dose of influenza A virus (H1N1- A/PR/8/34) infection. However, the lung virus titers were significantly higher in nicotine treated mice than that in the control mice [57]. In 2004, the same group further demonstrated that three weeks nicotine treatment decreased influenza A virus (H1N1-A/PR/8/34) induced lung inflammation, which was shown by the significantly reduced leukocyte accumulation in the lung, but in- creased the virus-specific mRNA expression in mice [58].

To further investigate the effect of nicotine on the immune response during influenza A virus infection, in our recent study entitled "Influenza virus-induced lung inflammation was modulated by cigarette smoke exposure in mice [13]" we investigated the risk of pdm-H1N1 and H9N2 influenza A virus infection after cigarette smoke exposure by using a mouse model. In order to study the underlying mechanism, we also used nicotine to mimic the cigarette smoke effect.

In this study, we exposed 6-8 weeks female C57/B6N mice to 4% cigarette smoke 4 hours one day for three weeks and then infected these mice with pdmH1N1 or H9N2 influenza A virus. We also exposed some other mice to fresh room air in parallel as the control. Our results showed that single cigarette smoke exposure could enhance the inflammation of lung significantly, as evidenced by the dramatically reduced body weight gain and up-regulated inflammatory response when compared with the control mice. The subsequent pdmH1N1 or H9N2 influenza A virus infection induced disease se- verity could be significantly decreased by such prior cigarette smoke exposure. After pdmH1N1 influenza A virus infection, the mice in cigarette smoke exposure group displayed a significantly reduced mortality com- pared to that in the control group, confirmed by the less secretion of cytokines and chemokines at day 5 post pdmH1N1 influenza A virus infection. In the same way, for H9N2 influenza A virus infection, the mice in ciga- rette smoke exposure group showed a milder disease significantly, which might be due to the lower body weight loss, weaker neutrophils, macrophages, $CD4^+$ and $CD8^+$ T cells infiltrating into the lung, less secretion of cytokines and chemokines, as well as decreased lung damage than that in the control group. However, cigarette smoke exposure had no effect on lung virus titer for both pdmH1N1 and H9N2 influenza A virus infection. These results identified that prior cigarette smoke exposure could ameliorate the pathogenicity caused by pdmH1N1 or H9N2 influenza A virus infection. The immunosuppressive effect of cigarette smoke exposure that decreased the influenza A virus stimulated excessive inflammatory response might be responsible for such beneficial effect. However, such effect had no effect on viral burden.

We further investigated the underlying mechanisms. As nicotine is one of the most important cigarette smoke components and has anti-inflammatory effect, we hypothesized that it was nicotine in cigarette smoke that had the possibility to be the key contributor for this immunosuppressive effect. The *in vitro* studies showed that for primary human macrophages, nicotine might significantly inhibit the secretion of IL-8, TNF-α and MIG after pdmH1N1 infection; and suppress the production of IL-8, TNF-α, MIG and RANTES after H9N2 virus infection. The *in vivo* experiments demon- strated that three weeks nicotine treatment significantly reduced the mouse body weight loss after both pdmH1N1 and H9N2 influenza A virus infection, which might be attributed to the less expression of some cytokines and chemokines in nicotine treated mice than in control mice. However, nicotine treatment had no effect on lung viral loads at specific time points after both pdmH1N1 and H9N2 influenza A virus infection. Importantly, our results also demonstrated that after H9N2 virus infection, cigarette smoke exposure could lead to significantly decreased body weight loss in wild-type mice than in α7 nAChR knockout mice. These *in vitro* and *in vivo* results further confirmed the anti-inflammatory effect of nicotine. Our study offered the first evidence that

the anti-inflammatory effect of nicotine in cigarette smoke might be the key contributor for the alleviation of the disease severity of both pdmH1N1 and H9N2 influenza A virus infection, and such anti-inflammatory effect was through the α7 nAChR signaling pathway.

Summary and perspective

This review article summarized the possible molecular mechanisms of the cholinergic anti-inflammatory pathway that induced the suppression of proinflammatory cytokines production via binding of nicotine to α7 nAChR. Moreover, all the recent findings related to the anti-inflammatory effect of nicotine were reviewed. Especially, we focused on the nicotine anti-inflammatory effect on influenza A virus infection. The anti-inflammatory effect of nicotine showed the protective role not only in H1N1-A/PR/8/34 infection in previous studies, but also in pdmH1N1 and avian H9N2 infection in our recent research.

The evidences of nicotine that inhibits the production of multiple cytokines via anti-inflammatory pathways are increasing and it is known that nicotine shows a protective role in various diseases through α7 nAChR. Consequently, nicotine might be used as a therapeutic in many diseases and disorders. For example, stimulating the α7 nAChR with its ligand nicotine generated optimal therapeutic advantage for Parkinson's disease, accompanied by a minimum of adverse side effects [59]. Nico- tine could be used as a drug in selected cases of acute ulcerative colitis [4]. The protective role of nicotine in influenza A virus infection also showed the potential therapeutic role of nicotine in decreasing the pathogenesis of highly virulent influenza A virus infection.

However, the side effects limit the therapeutic potential of nicotine. Nicotine has nonspecific binding effect. Except for the targeted organs or cells, it could also bind to other organs or cells and then be toxic [60]. The clinical study of nicotine therapy showed different levels of side effects due to the different routes and doses of nicotine administration. For example, in the clinical study of using nicotine as a treatment in ulcerative colitis, the most common side effects are nausea, dizziness, light-headedness, headache, tremor, sleep disturbance and contact dermatitis [4]. Such double-edged sword characteristics of nicotine make it difficult to predict its clinical application. Therefore, further studies are needed to improve the specificity of nicotine, investigate its associated adverse events and the minimum therapeutic dose to increase the therapeutic potential of nicotine.

Conflict of interest

The authors have declared that no competing interests exist.

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