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THE ANTI-INFLAMMATORY POTENTIAL OF SELECTIVE CHOLINERGIC AGONISTS

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The recent discovery of the anti-inflammatory potential of the vagus nerve has provided novel pharmacological targets to control lethal systemic inflammation in critical care (1, 2). Although the sympathetic system has been studied for years, only recent studies have revealed the anti-inflammatory potential of the parasympathetic system via the vagus nerve (1, 2). Different investigators have already shown that electrical or pharmacological stimulation of the vagus nerve restrains the production of inflammatory cytokines in experimental models of ischemia and reperfusion, hemorrhage and resuscitation, pancreatitis, colitis, endotoxemia, and polymicrobial sepsis. To avoid the clinical limitations of the vagus nerve stimulation, most of the studies focused on the cholinergic receptors mediating the anti-inflammatory potential of acetylcholine in immune cells. Acetylcholine, the principal neurotransmitter of the vagus nerve, and nicotine inhibit nuclear factor κ B (NF- κ B) and cytokine production in macrophages and splenocytes via the α 7 nicotinic acetylcholine receptor (α 7nAChR) (3, 4). *In vivo*, vagus nerve stimulation fails to inhibit LPS-induced serum TNF levels in α 7nAChR-knockout mice (3). Nicotine was previously used in clinical trials to reduce anxiety and improve cognition in schizophrenia, Alzheimer disease, and Parkinson disease, but its side effects have limited the clinical potential of this mechanism. Selective α 7nAChR agonists such as GTS21 were proven less toxic than nicotine by different laboratories and used in clinical trials for Alzheimer disease and schizophrenia. Among others, α 7nAChR agonists avoided the effects of nicotine to induce autonomic dysfunction, tachycardia, or arrhythmia. Epidemiological and clinical trials also indicated that cholinergic agonists such as nicotine can provide therapeutic anti-inflammatory potential in several inflammatory disorders including osteoarthritis and ulcerative colitis (1). Similar to that proposed in neurodegenerative disorders, α 7nAChR agonists were expected to avoid the adverse effects of nicotine and to provide pharmacological advantages to control inflammatory responses. This hypothesis is consistent with the specific role of particular cholinergic receptors in mediating specific physiologic and pharmacologic properties of acetylcholine and nicotine. Thus, α 7nAChR agonists are expected to avoid nicotine-induced addiction, locomotor activity, tumorigenesis, autonomic dysfunction, or allodynia, which appears to be mediated by other nicotinic receptors including β 2nAChR, α 3nAChR, α 4nAChR, or α 5nAChR.

In this issue, Kox et al. (5) analyzes the anti-inflammatory potential of the α 7nAChR agonist, GTS21, in human endotoxemia. This is the first study to investigate selective stimulation of the anti-inflammatory potential of the α 7nAChR in humans. Treatment with capsules, to the highest GTS21 dose tested in humans (450 mg/d), failed to induce consistent plasma levels of the compound or its metabolite (4OH-GTS21) and did not significantly affect the plasma levels of inflammatory cytokines in response to endotoxin (2 ng/kg i.v.) (5). However, the highest GTS21 plasma concentrations in the GTS21-treated subjects correlated with the lowest levels of TNF, IL-6, and IL-1RA. In similar settings of human endotoxin, previous studies indicated that transdermal nicotine (7 mg/patch) lowered the temperature response and increased the plasma levels of IL-10 and cortisol, but it did not

affect the plasma levels of inflammatory cytokines in response to endotoxin (2 ng/kg i.v.) (6). Unlike nicotine, GTS21 treatment did not affect serum levels of IL10 in a statistically significant manner. A typical limitation of these pilot studies is that the results in plasma cytokine levels may not be statistically significant because of the small group sample ($n = 7$ /group). In the present study, the authors have now estimated a sample size of $n \approx 61$ subjects/group will be necessary to achieve a power of 80%. Another significant limitation is the marked variability of both cytokine levels and GTS21 among the subjects. Similar limitations with the variability of the plasma levels of the drug or its metabolite (cotinine) were also reported in trials using transdermal nicotine (15 mg/d) to keep ulcerative colitis in remission. More significant effects were found with a steady release of higher concentrations of nicotine (25 mg/d) where the patches were worn all day long and not removed at bedtime in patients with acute colitis. In the present study, subjects ingested capsules of 150 mg GTS21 (t.i.d.) starting 3 days before the endotoxemia experiment. A crossover strategy, considering an appropriate washing period to prevent tolerance, higher doses, and alternative routes of administration such as intravenously, could ameliorate the variability among the subjects. To date, intravenous formulations for GTS21 are not yet available, and the highest dose tested in humans is 450 mg/d. The variability in plasma GTS21 levels between subjects was remarkable, even when the treatment was started 3 days before the experiment. Starting the treatment days before of the experiment may alleviate the variability of GTS21 in the subjects, but several concerns have been raised about the potential desensibilization of the nicotinic receptors. Isolated monocytes from treated and placebo subjects can be treated *in vitro* with GTS21 or other $\alpha 7$ nAChR agonists to evaluate the potential desensibilization of the nicotinic receptors. Previous studies already indicated that $\alpha 7$ nAChR agonists are quite efficient at inhibiting cytokine production in immune cells by inhibiting LPS-induced JAK2 activation and STAT3 tyrosine phosphorylation (7). Unphosphorylated STAT3 can bind to NF- κ B and prevent NF- κ B-induced TNF production. Likewise, GTS21 efficiently inhibited TNF production in human monocytes ($IC_{50} = 7 \mu M$), when they are isolated and treated *in vitro*, but it is not efficient when the whole blood treated *ex vivo* ($IC_{50} > 100 \mu M$). And yet, GTS21 is efficient *in vivo* including experimental models of neutrophil recruitment, murine lung inflammation, endotoxemia, polymicrobial sepsis, and hemorrhage and resuscitation. Treatment with GTS21 (4 mg/kg i.p.) started 30 min before endotoxemia dramatically inhibited serum TNF and HMGB1 levels and improved survival in both endotoxemia and cecal ligation and puncture in BALB/c mice (8). Resuscitation of hemorrhagic Sprague-Dawley rats with Hextend supplemented with GTS21 (0.2 mg/kg) inhibited NF- κ B and the production of inflammatory cytokines and improved organ function and survival (9). Together, these results suggest an alternative mechanism for the anti-inflammatory potential of GTS21 *in vivo*. Recent studies now indicate that the vagus nerve and $\alpha 7$ nAChR agonists control systemic inflammation in experimental sepsis by activating the splenic nerve to release norepinephrine via the $\alpha 7$ nAChR (10). Acetylcholine released by the vagus nerve in the celiac mesenteric ganglia activates postsynaptic $\alpha 7$ nAChRs of the splenic nerve leading to the release of norepinephrine in the spleen. Alpha7nAChR agonists induce the production of norepinephrine in the spleen in wild-type but not in $\alpha 7$ nAChR-knockout mice. Thus, the anti-inflammatory potential of $\alpha 7$ nAChR agonists is abrogated by splenectomy or splenic neurectomy (10).

This study of Kox et al. (5) provides a major advantage for the future design of pilot and clinical trials to analyze the anti-inflammatory potential of selective cholinergic agonists in infectious and inflammatory disorders. In addition to the new physiologic implications of the $\alpha 7$ nAChRs, these studies also propose the use of alternative $\alpha 7$ nAChR agonists that may provide pharmacokinetic advantages (1, 7, 10). GTS21 has been widely regarded as a selective $\alpha 7$ nAChR agonist, but its specificity and efficiency have been debatable. GTS21 has significant affinity for $\alpha 7$ nAChRs in rats, but very weakly efficacious at human $\alpha 7$ nAChRs. Its metabolite, 4OH-GTS21, was described as a selective partial agonist with at

least 10-fold greater efficacy for both human and rat $\alpha 7$ nAChRs than for any β -subunit containing nicotinic receptor. Novel $\alpha 7$ nAChR agonists such as choline, PNU282987, AR-R17779, and TC-7020 have shown promise in experimental models of inflammation, but have not been tested in humans yet. The new insights on the physiologic mechanisms modulating the immune system are giving us a better understanding of the inflammatory disorders associated with critical care and a novel perspective to design novel therapeutic strategies.

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