Neuro-immunity in intestinal disease: in vivo studies of postoperative ileus and colitis

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Selective alpha7 nicotinic acetylcholine receptor agonists worsen disease in experimental colitis
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Selective alpha7 nicotinic acetylcholine receptor agonists worsen disease in experimental colitis

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Abstract

**Background and aims:** IBD patients suffer from chronic and relapsing intestinal inflammation. In various models vagus nerve activation has been shown to ameliorate intestinal inflammation, via nicotinic acetylcholine receptors (nAChRs) that are expressed on immune cells. As the α7 nAChR has been put forward to mediate this effect, we studied the effect of nicotine, and two selective agonists for the α7nAChR (AR-R17779 and GSK1345038A) on disease in two mouse models of experimental colitis.

**Methods:** Colitis was induced by 1.5% dextran sodium sulfate (DSS) in drinking water or 2 mg 2,4,6-trinitrobenzene sulfonic acid (TNBS) intrarectally. Nicotine (0.25 and 2.5 μmol/kg), AR-R17779 (0.6–30 μmol/kg) and GSK1345038A (6–120 μmol/kg) was administered daily by intraperitoneal injection. After seven (DSS) or five (TNBS) days clinical parameters and colonic inflammation were scored.

**Results:** Nicotine, as well as both selective α7 nAChR agonists reduced the activation of NF-κB and pro-inflammatory cytokines in whole blood and macrophage cultures. In DSS-induced colitis, nicotine treatment reduced colonic cytokine production, but failed to reduce disease parameters. Reciprocally, treatment with either α7 nAChR agonist AR-R17779 or GSK1345038A worsened disease parameters and led to increased colonic pro-inflammatory cytokine levels in DSS colitis. Only the highest doses of GSK1345038A (120 μmol/kg) and AR-R17779 (30 μmol/kg) ameliorated clinical parameters, albeit without affecting colonic inflammation. Neither agonist ameliorated TNBS induced colitis.

**Conclusions:** Although nicotine reduces cytokine responses in vitro, both selective α7 nAChR agonists worsen DSS colitis or are ineffective in TNBS colitis. Our data warrant caution in evaluating α7 nAChR as a drug target in colitis.
Introduction

Genetic association studies (1) and functional evidence (2;3) has increased the recognition that intestinal macrophages play an important role in initiation and progression of inflammatory bowel disease (IBD). In several studies it was demonstrated that resident macrophages in mucosal samples of active ulcerative colitis (UC) and Crohn’s disease (CD) patients differ phenotypically and functionally from healthy controls (4-6). Similarly, data obtained from mouse models of colitis imply an important role for macrophages: in IL-10 deficient mice that develop colitis spontaneously, intestinal inflammation is prevented by the use of antagonists of chemokine receptors (7) that are generally expressed by macrophages, or by elimination of tissue macrophages (3). Furthermore, colitis can still be induced in the absence of T and B cells (2). Recently, it has been shown that macrophage derived IL-10 is crucial for the induction of regulatory T-cells, thereby controlling intestinal inflammation in colitis (8).

Recently, the parasympathetic system, in particular the vagus nerve, has been shown to negatively regulate macrophage immune responses via the peripheral release of acetylcholine (ACh) (9;10). Activation of the so-called ‘cholinergic anti-inflammatory pathway’ has been shown to ameliorate disease in various models of inflammatory disease including, sepsis (9), ischemia reperfusion (11), hemorrhage (12) and postoperative ileus (13). In mouse models of colitis and postoperative ileus, enhanced parasympathetic output is involved in the negative regulation of intestinal inflammation via efferent activity of the vagus nerve (13-15). Ghia et al. have recently demonstrated that the vagus nerve controls gut inflammation in two experimental models of colitis (14;15). In these studies it was shown that chemical as well as surgical blockade of vagus nerve signaling significantly worsens colitis and enhances colonic inflammatory mediators. The vagus nerve anti-inflammatory effect most likely involves activation of the nicotinic acetylcholine receptors (nAChRs) on immune cells such as macrophages (9;13;16;17) or dendritic cells (18;19). This notion is supported by clinical observations that smoking, and the administration of nicotine (i.e. via patches) may have a protective effect on colonic inflammation in UC, even though results are generally disappointing due to the significant toxic adverse-events (20).

The cellular pathways of nicotinic inhibition of macrophage activation involve the activation of anti-inflammatory Stat3/Socs3 signaling pathways (13) and inhibition of NF-κB signaling (21). Earlier studies indicate that the anti-inflammatory effect of acetylcholine is mediated through the α7 nicotinic acetylcholine receptor (α7 nAChR) (9;17) expressed by human (17;21) and mouse macrophages (17;18;21). Given the purported role of α7 nAChRs in mediating the cholinergic anti-inflammatory pathway (13;17;22), selective α7nAChR agonists may bear more therapeutic potential in ameliorating disease compared to nicotine. Therefore, we explored the potential of pharmacological activation of the cholinergic anti-inflammatory pathway by treatment with nicotine and two α7 nAChR selective agonists in two mouse models of colitis. In dextran sodium sulfate (DSS) induced colitis, our results show that nicotine does not affect disease severity. Both selective α7 nAChR agonists AR-R17779 and GSK1345038A affect disease severity in a bell-shaped response curve; low doses aggravate disease, while high doses ameliorate disease. In 2,4,6-trinitrobenzene sulfonic acid (TNBS) colitis, treatment with GSK1345038A was ineffective. These data have important repercussions on the therapeutic potential of selective α7 nAChR agonists in colitis.
Material and Methods

Animals
Female C57BL/6 mice (8-10 wk old and weighing 20-25 g; Charles River) were housed and maintained under standard conditions at our animal facility. Food and water were given ad libitum. All animal experiments were performed according to the guidelines of the Animal Research Ethics Committee of the University of Amsterdam.

Induction of colitis.
To induce DSS colitis, 1.5% (w/v) DSS (TdB Consultancy, Uppsala, Sweden) was administered in the drinking water of the mice during 7 days. Body weight was recorded daily and weight loss as on day 7 as compared to day 0 was calculated. Animals were killed on day 7 of DSS administration. Hapten-induced colitis was induced by rectal administration of one dose of 2 mg TNBS (Sigma Chemical Co, St Louis, MO) in 40% ethanol (Merck, Darmstadt, Germany), using a vinyl catheter that was positioned 3 cm from the anus. During the instillation, the mice were anesthetized using isoflurane (Abbott Laboratories Ltd., Queenborough, Kent, UK), and after the instillation mice were kept vertically for 30 sec. Five days after TNBS instillation, mice were killed. Mice received a daily intraperitoneal (i.p.) injection with nicotine (0.25 or 2.5 μmol/kg) (Sigma-Aldrich, Zwijndrecht, the Netherlands); AR-R17779 ((−)-Spiro[1-azabicyclo[2.2.2]octane-3,5’-oxazolidin-2’-one)(0.6-30 μmol/kg) (kindly provided by Critical Therapeutics, Lexington, MA) or GSK1345038 (6-120 μmol/kg) (kindly provided by Glaxo SmithKline, Stevenage, UK) in 1% methylcellulose. The treatment with the agonists was started at the first day of DSS administration.

GSK1345038A pharmaco-kinetics.
60 or 120 μmol/kg of GSK1345038A was administered i.p. to C57Bl/6 mice (n = 4) and blood samples were taken at time points 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8 and 12 h. Blood samples were analyzed for the free base of GSK1345038A using a method based on protein precipitation and HPLC-MS/MS analysis. To samples of blood (50 μL diluted with 50 μL with water), acetonitrile: ammonium acetate (10mM) (8:2, 250 μL) containing an appropriate internal standard was added. Samples were mixed thoroughly (mechanical shaking for 20 min), and then centrifuged (2465 x g for 15 min at room temperature). An aliquot of the resulting supernatant was analyzed for GSK1345038A by reverse phase HPLC-MS/MS using a heat assisted electrospray interface in positive ion mode (Sciex API 4000) and a ACE-3 C18 column (50 x 4.6mm ID, 3um; Hichrom). The mobile phase was delivered as a linear gradient of 20% to 95% acetonitrile:ammonium acetate (1mM containing 0.1%v/v formic acid) over 0.8 min. The final composition was held for 0.8 minutes before return to initial composition. Nominal MRM transition for GSK1345038A was 454 to 123. Concentration range for the assay was; 0.011 to 22.0 μM with a lower limit of quantification (LLQ) of 0.011 μM.

Assessment of colitis
Faecal blood, diarrhoea and disease activity index (DAI) were scored as described in table 1. The wet weight of each colon was recorded and used as an index of disease-related intestinal wall thickening. The total length of the colon was measured and colon shortening as a consequence of DSS- induced colitis was used as a disease parameter. Subsequently, the colons were separated from mesentery and fat and longitudinally divided into two parts for histological examination and measurement of cytokines.
Histological examination
The longitudinally divided colons were fixed in 4% formalin and embedded in paraffin for routine histology. An experienced pathologist microscopically evaluated formalin-fixed haematoxylin tissue sections in a blinded fashion. Rolled colon was evaluated, and graded from 0 to 26 points as indicator of incidence and severity of inflammatory lesions based on the extent of the involved area, the number of follicle aggregates, oedema, fibrosis, hyperplasia, erosion/ulceration, crypt loss, and infiltration of granulocytes and mononuclear cells (table 2).

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Table 2: Histopathology scoring.

Colonic cytokine production
For cytokine measurements, colons were diluted 1:9 in lysis buffer containing 300 mM NaCl, 30 mM Tris, 2 mM MgCl2, 2 mM CaCl2, 1% Triton X-100, pepstatin A, leupeptin, and aprotonin (all 20 ng/ml; pH 7.4), and incubated at 4°C for 30 min. Homogenates were centrifuged at 1500 x g at 4°C for 15 min, and supernatants were stored at -20°C until analyses. TNF, IL-6 and IL-17 in supernatants were analyzed by mouse ELISA Duoset kits (R&D Systems, Minneapolis, MN). Assays were performed according to the manufacturer’s instructions.

Whole blood stimulation assays
Whole blood was taken via heart puncture following anaesthesia. Aliquots of 50 μl were divided onto round bottom 96 wells plates and treated with appropriate concentrations of nicotinic
agonists diluted in 50μL of RPMI 1640 supplemented with 10% heat-inactivated foetal calf serum (FCS; Gibco-BRL, Breda, The Netherlands), 2 mM L-glutamine, 1000 U/ml penicillin, 1000 μg/ml streptomycin, 250 ng/ml amphotericin B (Gibco) for 15 min 37°C. Subsequently, heat killed E. coli (1x10⁴/well) or LPS (Sigma) at a final concentration of100 ng/ml was added to the wells. After 3 h of stimulation, plates were centrifuged, supernatants were collected and levels of TNF, IL-6 and IL-17 were analyzed by ELISA (R&D Systems).

NF-κB activity assay
Immortalized peritoneal macrophages RAW264.7 were stably transfected with a NF-κB luciferase reporter construct (Clontech, Mountain View, CA) in which a PDNA3.1(+) derived neomycin resistance TK cassette was inserted (referred to as pNF-κBneo-luc). Transfection was performed using Nucleofactor V (Lonza, Cologne, Germany). Briefly, 0.5 μg per 106 cells of constructs pNF-κBneo-luc was suspended in 75 μl of 150mM sterile NaCl solution. The transfection was allowed to proceed for 16 h, and the medium refreshed. Twenty-four h after transfection, neomycin resistant clones were selected and subcloned. For assay, cells were pre-treated with nicotinic agonists at the concentration indicated for 20 minutes, washed and subsequently stimulated with LPS (100ng/mL; Sigma) for 6 h. After treatment, the medium was removed; the cells washed three times with ice-cold PBS and cells lysed with Passive Lysis Buffer supplied in the LuciferaseTM Reporter Assay Kit (Promega Corporation, Madison, WI) and the lysate was assayed for luciferase activity according to the manufacturer’s instructions.

Statistics
The values of the clinical parameters of the groups without DSS and TNBS groups treated with nicotine and α7 nAChR agonists are relative values (%) as compared to the DSS and TNBS groups treated with vehicle. Differences between groups were analyzed using the nonparametric Mann-Whitney U test. P<0.05 was considered significant. All analyses were performed using SPSS (SPSS Inc. Chicago, Ill, USA)

Nomenclature
All drug/molecular target are termed in accordance with BJPs Guide to Receptors and Channels (23).

Results
Macrophage activation is modulated by nicotine, and α7 nAChR agonists GSK1345028A and AR-R17779.
First, we reproduced that nicotine, AR-R17779 (13;24), and GSK1345028A reduced TNF and IL-6 release in-vitro in Biogel elicited peritoneal macrophages stimulated with heat-killed E. coli or LPS in a 0 -10μM concentration range (24)(and data not shown). In line with these previous observations, AR-R17779 and GSK1345028A, as selective α7 nAChR agonists, were less potent in reducing peritoneal macrophage cytokine release as compared to nicotine (24) (and data not shown). In whole blood stimulations (Fig. 1), nicotine or α7 nAChR agonists AR-R17779 and GSK1345028A significantly reduced IL-6 and TNF production in LPS—or heat-killed E. coli activated whole blood cell preparations, albeit the potency to reduce cytokine production was less pronounced. None of the three agonists significantly reduced IL-6 production after stimulation with LPS (Fig. 1). Values for non-stimulated cells were below detection (data not shown).
The potential of nicotinic agonists to reduce cytokine production has previously been associated with inhibition of NF-κB activity (22;24). We explored the potency of GSK1345028A and AR-R17779 to reduce NF-κB transcriptional activity in activated macrophages. To this end, we investigated the effect of nicotine, AR-R17779 and GSK1345038A on NF-κB activation induced by LPS in a reporter assay using macrophage cell line RAW264.7 which was stably transfected with a NF-κB reporter construct. As shown in Fig. 2, LPS induced NF-κB transcriptional activity that was significantly reduced by nicotine, AR-R17779, as well as GSK1345038A.
Treatment with nicotine does not affect clinical parameters in DSS-induced colitis.

Given the reported potential of the vagus nerve to reduce disease in various mice models, including colitis (14;15), and the positive association of smoking with the course of UC (25) we next tested whether treatment with nicotine affected the disease course of DSS–induced colitis. Daily treatment with nicotine did not alter weight loss (Fig. 3A) or DAI (Fig. 3B) as compared to vehicle treated group. Only colon weight, which represents thickening of the colon by oedema, was significantly reduced by treatment with both 0.25 and 2.5 μmol/kg nicotine (Fig. 3C) but colon shortening was not affected by nicotine administration (Fig. 3D). To test the effect of nicotine on intestinal inflammation we measured the production of TNF, IL-6 and IL-17 in colon homogenates. Although TNF levels were not altered, colonic IL-6 and IL-17 levels were significantly reduced by nicotine treatment (Fig. 3). However, this reduced cytokine production was not reflected in a decreased histopathology score (Table 3).
Figure 3. Effects of nicotine on DSS-induced colitis.

(A) % Body weight on day 7 as compared to body weight on day 0 of the experiment. (B) Disease activity index (DAI) as described in material and methods. (C) Colon weight per cm colon. (D) Colon length. (E) TNF, IL-6 and IL-17 levels in colon homogenates. Data are expressed as % of mice receiving DSS and treated with vehicle. Asterisks indicate significant differences (* P<0.05, ** P<0.01) as compared to DSS group treated with vehicle. n = 10 per group. Bars indicate mean ± SEM.
Treatment with α7 nAChR agonists GSK1345038A and AR-R17779 worsens clinical parameters of colitis.

We next questioned whether nicotine treatment failed to ameliorate disease in DSS-induced colitis because nicotine does not selectively target the α7 nAChR. In separate experiments, we therefore tested the efficacy of AR-R17779 and GSK1345038A in DSS-induced colitis. A dose of 6 μmol/kg of AR-R17779 results in a Cmax of 4.6 μM and a half life of approximately 150 min (G.R., personal communication), and should thus reach the effective concentration range to reduce cytokine release in macrophages (24) and whole blood (Figs. 1 and 2). Hence we dosed AR-R17779 at a 1.8-30 μmol/kg dose daily. Daily i.p. injection with 1.8, 6 and 18 μmol/kg with the α7 nAChR agonist AR-R17779 aggravated weight loss (AR-R17779: 1.8 μmol/kg: 88.7 ± 1.1%; 6 μmol/kg: 95.0 ± 1.1%; 18 μmol/kg: 95.0 ± 1.1% relative to vehicle group). In contrast, in the group treated with a highest dose of AR-R17779 (30 μmol/kg) weight loss was prevented (107.4 ± 1.9 % relative to vehicle treated group) (Fig. 4A).

To confirm these data, we next tested another selective α7 nAChR agonist, GSK-1345038A in the same model of DSS-induced colitis. Similar to AR-R17779, we first assessed the optimal dosage range for GSK1345038A by measurement of the blood concentration of GSK1345038A. The pharmacokinetics indicated that GSK1345038A has a half live of 2-3hrs, and reaches blood concentrations of 5-25 μM in a dosage range of 60-120 μmol/kg mouse (Fig. 5), i.e. the effective dose range to reduce cytokine release in our in vitro assays (Figs. 1 and 2). Hence, to reach optimal circulation levels in vivo, we administered doses of 6, 20, 60, and 120 μmol/kg in daily i.p. injection protocol. In line with the results obtained using AR-R17779, weight loss was significantly enhanced by daily injection with 6, 20 or 60 μmol/kg GSK1345038A (GSK1345038A: 6 μmol/kg: 87.9 ± 1.1%; 20 μmol/kg: 92.1 ± 1.5%; 60 μmol/kg: 90.4 ± 1.9 % relative to vehicle group) (Fig. 4A). In correspondence to the effect of the highest dose of AR-R17779 on colitis course, weight loss was prevented by daily treatment with the highest dose of GSK1345038A (120 μmol/kg) tested (103.1 ± 1.9 % relative to vehicle group) (Fig. 4A).

The effects of both α7 nAChR agonists on disease activity index (DAI) paralleled those of the effects on weight loss as treatment by AR-R17779 significantly enhanced DAI (AR-R17779: 1.8 μmol/kg: 150.5 ± 15.9%; 6 μmol/kg: 143.9 ± 9.2% and 18 μmol/kg: 128.0 ± 8.0%) (Fig. 4B). In contrast, DAI was significantly reduced after treatment with the highest dose of 30 μmol/kg AR-R17779 (73.5 ± 6.9%) (Fig. 4B). Similar results were obtained by treatment
with GSK1345038A that significantly worsened disease as reflected in DAI (GSK1345038A: 6 μmol/kg: 131.3 ± 8.2%; 20 μmol/kg: 135.4 ± 12.5%; 60 μmol/kg: 154.2 ± 9.9%), except for the highest dose of 120 μmol/kg GSK1345038A, that ameliorated DAI (68.7 ± 6.5%) compared to vehicle (Fig. 4B). In contrast to nicotine treatment, the increase of colon weight was unaffected by either of the α7 nAChR agonists (Fig. 4C), while the DSS-induced decrease in colon length was further reduced by AR-R17779 and treatment with GSK1345038A was ineffective (Fig. 4D).

Figure 4. Effects of the α7 nAChR agonists AR-R17779 and GSK1345038A on DSS-induced colitis. (A) % Body weight on day 7 as compared to body weight on day 0 of the experiment. (B) Disease activity index (DAI) as described in material and methods. (C) Colon weight per cm colon. (D) Colon length. Data are expressed as % of DSS group treated with vehicle. Asterisks indicate significant difference (* P < 0.05, ** P < 0.01, *** P < 0.001) as compared to DSS group treated with vehicle. Mice per group: 0.6, 1.8, 6, 18, µmol/kg AR-R17779 and 6, 20, 60 µmol/kg GSK1345038A: n = 10; vehicle groups and 120 µmol/kg GSK1345038A: n = 18. Bars indicate mean ± SEM.
The effect of the α7 nAChR agonists GSK1345038A and AR-R17779 on colonic inflammation in DSS-induced colitis

We next measured the effect of α7 nAChR agonists on colonic cytokine production after 7 days of DSS administration. In line with the augmented disease outcome, nicotine treatment (Fig. 3E), but neither of the α7 nAChR agonists reduced colonic TNF and IL-6 (Fig. 6). In contrast, colonic TNF, IL-6, and IL-17 were significantly elevated after treatment with AR-R17779, but not GSK1345038A (Fig. 6).

Figure 5. Blood time course concentrations of GSK1345038A. 60 (closed circles) or 120 µmol/kg of GSK1345038A (open circles) was administered i.p. to C57Bl/6 mice. Concentrations of GSK1345038A was measured in mouse blood was assessed at indicated time points. Data shown are the mean ± SEM of triplicate measurements of 4 mice.

Figure 6. TNF, IL-6 and IL-17 production in the colon.
Effects of treatment with AR-R17779 and GSK1345038A at the indicated dose on colonic cytokine production. Asterisks indicate significant differences (* P < 0.05) as compared to vehicle. Agonist treated groups: n = 10; vehicle groups and 120 µmol/kg GSK1345038A: n = 18. Data are expressed as the mean ± SEM.
In addition, histopathology scores were assessed for the doses of the \(\alpha_7\) nAChR agonists with most pronounced effects on disease severity. As indicated in Table 4, total histopathology scores after treatment with 30 \(\mu\)mol/kg AR-R17779 and 120 \(\mu\)mol/kg GSK1345038A did not parallel clinical scores as there was no significant difference between groups. Similar effects were observed by administration of lower doses of AR-R17779 (1.8 \(\mu\)mol/kg) and GSK1345038A (60 \(\mu\)mol/kg); clinical outcome was poorer, but total histopathology scores were not significantly different from vehicle controls, except for crypt loss that was significantly worsened by treatment with 1.8 \(\mu\)mol/kg AR-R17779 and 60 \(\mu\)mol/kg GSK1345038A dosage (Table 4).

**Table 4. The effect of \(\alpha_7\) agonists AR-R17779 and GSK1345038A on colonic inflammation in DSS-induced colitis.**

C57BL/6 mice were administered 1.5% DSS in drinking water and killed at day 7. H&E stainings were performed on whole colons including rectum from groups treated with vehicle, 1.8 and 30 \(\mu\)mol/kg AR-R17779 or 60 and 90 \(\mu\)mol/kg GSK1345038A and scored by an experienced pathologist. H&E stainings of colon and rectum showed inflammatory features including crypt damage, follicles, edema, ulceration and influx of inflammatory cells. Asterisks indicate significant differences (* \(P < 0.05\)) as compared to vehicle. Mice per group: Vehicle: \(n = 18\); 1.8 \(\mu\)mol/kg AR-R17779 and 60 \(\mu\)mol/kg GSK1345038A: \(n = 10\); 120 \(\mu\)mol/kg GSK1345038A: \(n = 18\). Data represent mean ± SEM.

The effect of the \(\alpha_7\) nAChR agonist GSK1345038A in a mouse model of acute TNBS colitis

To investigate whether the effects of \(\alpha_7\) nAChR agonists on colitis were selectively observed in the acute DSS colitis model, we tested GSK1345038A in another acute model of colitis, TNBS induced colitis. The main read out for this model is colonic inflammation and not clinical parameters as the mice are allowed to recover after one dose of TNBS. As indicated in Fig. 7A, weight loss 5 days after instillation of TNBS was not significantly different between groups treated with GSK1345038A and vehicle. In addition, histopathology scores were not significantly altered by treatment with 60 \(\mu\)mol/kg and 120 \(\mu\)mol/kg GSK 1345038A (Table 5). GSK1345038A treatment did not alter colonic production of TNF and IL-17, while IL-6 production was below detection (Fig. 7B).
Figure 7. Effect of the α7 nAChR agonist GSK1345038A on TNBS-induced colitis. (A) Body weight is indicated as percentage of body weight on day 0 of the experiment (B) Cytokine levels in colon homogenates. Asterisks indicate significant differences (* \( P < 0.05 \)) as compared to vehicle. Bars indicate mean ± SEM, \( n = 7 \).

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Table 5. The effect of α7 agonists AR-R17779 and GSK1345038A and on colonic inflammation in TNBS induced colitis. Mice (\( n = 7 \) per group) received one dose of 2 mg TNBS in 30 % ethanol intrarectally and were killed after 5 days. Vehicle, 60 or 120 µmol/kg GSK1345038A or was injected daily. H&E stainings were performed on whole colons including rectum and scored by an experienced pathologist. H&E stainings of colon and rectum showed inflammatory features including crypt damage, follicles, edema, ulceration, hyperplasia and influx of inflammatory cells. Asterisks indicate significant differences (* \( P < 0.05 \)) as compared to vehicle. Data represent mean ± SEM.
Discussion

IBD patients suffer from chronic and relapsing intestinal inflammation, initiated by aberrant responses of the innate immune system (26;27). Recently, a number of animal studies demonstrate that innate immune responses are attenuated by stimulation of the efferent arm of the vagus nerve through its neurotransmitter ACh, that acts on nAChRs, in particular the α7 nAChR, on resident macrophages (9;17). In various mouse models of inflammatory disease, we (13;24;28) and others (17;21), observed anti-inflammatory effects of vagus nerve stimulation, as well as pharmacological stimulation of the cholinergic system by administration of nicotine and α7 nAChR agonists. In the current study, we aimed to extend these studies by treating experimental colitis through targeting α7 nAChRs with nicotine, and two selective α7 nAChR agonists AR-R17779 and GSK1345038A. The agonists were tested in two mouse models of acute colitis: DSS and TNBS induced colitis. In vitro, nicotine reduces macrophage NF-κB activity and cytokine release significantly. In addition, treatment of DSS-induced colitis with nicotine led to a significant reduction in colonic oedema and colonic IL-6 and IL-17 production. However, this reduction was not enough pronounced to be reflected in clinical parameters and histopathology scores. The histopathology scores are the endpoint of the inflammatory reaction and contribute greatly to the functionality of the colon and thus have a large influence on clinical outcome. In addition, reduced IL-17 levels do not strictly imply reduced disease activity and it has been recently described that IL-17 might act as an anti-inflammatory cytokine in the gut (29).

UC Patients with a history of smoking usually acquire their disease after they have stopped smoking (30-32). Patients who smoke intermittently often experience improvement in their colitis symptoms during the periods when smoking (25;31;33). Following this reasoning and given the previous reports on the positive effect of cholinergic activation in experimental models of DSS colitis (14;15), nicotine treatment may well be beneficial in UC. Indeed, in patient studies treatment with transdermal nicotine was effective for the induction of disease remission in UC patients (20), but the number of patients that suffered from adverse effects was significantly higher in the nicotine treated patient groups (20) as compared to patients treated with standard therapy. Of note is that smoking in CD patients worsens disease.

In the current study we tested, besides nicotine, two selective α7 nAChR agonists, AR-R17779 (34) and GSK1345038A, in the mouse model of DSS and induced colitis. Treatment with the α7 nAChR agonists both displayed a bell-shaped dose response curve; the highest doses of AR-R17779 and GSK1345038A significantly ameliorated clinical parameters, as lower doses of both compounds worsened or did not affect clinical parameters. The highest doses used ameliorated clinical outcome but did not affect inflammatory parameters. Although our data confirm the capacity of AR-R17779 and GSK1345038A to reduce pro-inflammatory mediator release in vitro in macrophage cultures (24) and whole blood, the reduction in cytokines as well as activation of NF-κB induced genes was around 20-40%, which proved not to affect disease outcome in the colitis models used in this study.

However, a possibility exists that at the highest dose, the α7 nAChR agonists might show off target activity and lose their selectivity for the α7 nAChR, thereby affecting disease in an α7 nAChR independent fashion. We should keep in mind that nAChRs are expressed peripherally as well as centrally and that activation of nAChR on neurons can have analgesic effects, or modify mucus production, gut motility and blood flow to the gut (25;35). In the DSS colitis model, these effects might control food intake and formation of stools thereby influencing disease activity parameters independently of the severity of colonic inflammation. Another effect of nAChR activation can be the change in muscle tone thereby reducing colon length.
This might play a role in the significant reduction of colon length we observed by treatment with AR-R17779.

In addition, activation of nAChRs plays a role in regulating epithelial permeability (35;36) and bacterial clearance (37;38), important factors in the development of colitis which were not assessed in our experiments. Thus, nAChR activation can have a variety of effects on disease, independent of immune mediation, because of its widespread expression on different cell types as well as on different tissue types.

Although we report here that treatment with nicotine, or selective α7 nAChR agonists, is not effective in experimental colitis, enhanced vagus nerve output has been shown to reduce inflammation in various mouse models (9;24;37;39-41). This cholinergic anti-inflammatory effect seems to rely on the expression of the α7 nAChR on innate immune cells (9;17). Reciprocally, in colitis mouse models, it has been shown that vagotomy worsens colitis, an effect that was shown to be counteracted by nicotine administration (14;15;42). Of note in the interpretation of these studies is that the vagus nerve only marginally innervates the distal colon, making direct effects of ACh on colonic immune cells unlikely. Probably, the vagus nerve relays its immune modulatory effects to the colon in an indirect fashion, i.e. via postganglionic activation or by targeting alternative cell types. Of interest in this respect is a more recent study in which vagotomy was shown to worsen DSS colitis due to an impaired potential of antigen presenting cells (APCs) to induce regulatory T-cells (42). Notably, the physiological effects of vagus nerve stimulation or vagotomy as compared to pharmacological activation of ACh receptors differ greatly, especially when taking into account the changes in sympathetic output. In addition, vagus nerve stimulation or vagotomy will not only target nAChRs, but also influence the release of a number of neurotransmitters in the gut that regulate immune functions, and gut functions such as permeability and blood flow that possibly influence disease outcome.

Irrespective however, nicotine administration ameliorated disease in previous studies of experimental colitis (14;43). We cannot explain why the effectiveness of nicotine to reduce disease parameters was less pronounced in our study. The nicotine dose used in this study was effective to reduce inflammation in other models of inflammation (24;28), but possibly, in our colitis experiments a higher dose is required. However, in mice, higher dosage would result in adverse effects because of activation of a broad range of receptors both peripherally and centrally (44). In addition, a large array of nAChRs subtypes are expressed (45), and previous studies point towards a role in modulation of intestinal inflammation for nAChRs containing α5 (46) or α4β2 (18). Thus, the nAChR subtype involved in the immunomodulatory properties of the vagus nerve remains to be established.

Alternatively, the outcome of animal experiments with nAChR agonists could be dependent on the model of inflammation studied, as expression of the nAChR might vary depending on tissues and cell types involved in disease development. Notably, in other studies, nicotine treatment worsened the course of jejunitis in rodent models (47), and TNBS colitis (48). There are notable differences amongst colitis models (49), which might be important in the effectiveness of the administered agents. Thus, the effects of nicotine and α7 nAChR agonists may depend on many experimental factors such as the dose used, administration method, disease severity and disease model used.

We conclude that in developing a strategy for treating colitis using cholinergic agonists we should keep in mind that expression of nAChR is extremely widespread centrally and peripherally. In addition, expression of nAChRs subtypes on target cell types has to be carefully investigated before evaluating nAChRs α7 as drug target in colitis patients.
Reference List


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