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REVIEW ARTICLE

The effect of smoking on intestinal inflammation: What can be learned from animal models?

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Abstract

Epidemiological evidence demonstrates that smoking is the most important environmental risk factor in Crohn's disease while it positively interferes with the disease course of ulcerative colitis. However, the underlying mechanisms through which smoking exerts this divergent effect and affects pathogenesis of inflammatory bowel disease are largely unknown. Animal smoke models are good models to investigate the impact of cigarette smoke on intestinal physiology and inflammation. They enable one to explore the interaction of smoke components and the gut on cellular and molecular level, clarifying how smoking interferes with normal gut function and with disease course in inflammatory conditions. This review describes the currently used animal models for studying the impact of cigarette smoke on the intestinal tract. We first discuss the different methods for simulation of smoking. Furthermore, we focus on the effect of smoke exposure on normal gut physiology and immunology, on experimental (entero)colitis, and on inflammation-induced neoplasia. Based on this current knowledge, a hypothesis is formulated about the mechanisms through which cigarette smoke interferes with the gut in normal and pathological conditions.

Abbreviations: CD, Crohn's disease; CO, carbon monoxide; COX, cyclooxygenase; DAI, disease activity index; DC, dendritic cell; DNBS, 2,4-dinitrobenzene sulfonic acid; DSS, dextran sulfate sodium; FAE, follicle-associated epithelium; GSH, glutathione; IBD, inflammatory bowel disease; IL, interleukin; iNOS, inducible nitric oxide synthase; ITF, intestinal trefoil factor; LT, leukotriene; MPO, myeloperoxidase; nAChR, nicotinic acetylcholine receptor; PG, prostaglandin; ROMs, reactive oxygen metabolites; SOD, superoxide dismutase; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TNBS, trinitrobenzene sulfonic acid; TNF, tumor necrosis factor; Treg, regulatory T-cell; UC, ulcerative colitis; XO, xanthine oxidase.

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1. Introduction

The relationship between smoking and inflammatory bowel disease (IBD) is well recognized since many years. Cigarette smoke is considered to be the most important environmental risk factor in IBD pathogenesis. The first reports underscoring its role in IBD date from 30 years ago.[1,2] Since then, a large number of epidemiological studies described the dual effect of (active) smoking: it is associated with a higher risk for developing Crohn's disease (CD) and a worse outcome in CD patients, whereas ulcerative colitis (UC) is primarily a disease of non-smokers and smoking and nicotine are assumed to play a protective role in UC.[3,4] The ethnic background of patients also interferes with the effect of smoking on IBD. Most studies were performed in a Caucasian population; however, several epidemiological studies performed in Israel failed to find an association between smoking and Crohn's disease in Jewish patients.[5,6] More recently, also passive smoking was investigated as potential risk factor for IBD. However, a large meta-analysis in 2008 could not demonstrate an association between passive smoking and the incidence of CD or UC.[7]

The numerous descriptive and epidemiological reports on smoking and IBD are in sharp contrast with the limited amount of mechanistic studies exploring the effect of cigarette smoke on the gut and on intestinal inflammation in particular. The molecular and cellular mechanisms by which smoking interferes with the pathogenesis of CD and UC are only partially understood. Although several potential mechanisms, such as modulation of mucosal immune responses, alterations in intestinal cytokine and eicosanoid levels or modifications in gut permeability have been proposed, none of these hypotheses could offer a satisfying explanation.[8,9] Moreover, the exact component(s) of cigarette smoke exerting the dual effect on CD and UC is not identified up till now. Tobacco smoke contains more than 4500 chemicals, many of them being toxic or interfering with the immune system. Among these, nicotine, carbon monoxide (CO) and nitrogen oxide are known to have immunomodulatory capacities and are possible mediators in IBD pathogenesis, but also other tobacco constituents can interact with intestinal immunity and gut function.[10]

One of the problems the scientific world is facing in exploring the interaction between smoking and the gut, is the difficulty to find a good model. On one hand, human studies are complex and establishing a good study design with sufficient power is demanding. Firstly, definitions of who is considered a smoker or non-smoker are hard to make, and smoke anamnesis is not always reliable. Furthermore, numerous potential confounding factors have to be dealt with, such as medication and social class. On the other hand, in vitro studies in which cigarette smoke extract or smoke constituents are administered to intestinal cell cultures or cells isolated from IBD patients can be used. This kind of experiment is easier to standardize and interfering factors can be better controlled. However, in vitro experiments stand far away of clinical reality. Findings of these studies, although valuable, have to be interpreted conservatively. A third possibility to investigate the relationship between smoking and IBD is the use of animal models. These offer the advantage of performing in vivo experiments in easier controllable conditions than human studies. Although human active smoking is difficult to mimic in laboratory conditions, several animal models exist using smoke exposure or nicotine administration.

Despite the important role of smoking in pathological conditions in the gut such as IBD and colorectal carcinoma,[11,12] only a limited number of groups have applied

these animal smoke models. In this review, we discuss the different established animal smoke models used in intestinal research, focusing on the different methods for simulation of smoking and on the smoke-induced effect on healthy gut tissue, intestinal inflammation and inflammation-associated neoplasia. Finally we reflect on the mechanism(s) through which smoking might interact with the small bowel and colon in normal and inflammatory conditions.

2. Animal models investigating the effect of smoke exposure on the gut

Several groups investigated the effect of cigarette smoke or its components on normal jejunum, ileum and colon in animal models. A wide range of methods has been applied (Table 1).

2.1. Nicotine

A number of groups studied the effect of nicotine, assuming that nicotine is the main active metabolite responsible for the impact of smoking on the course of IBD.[9] A wide range of administration methods and doses are applied in the models described below, making it difficult to compare these studies with one another.

The best studied way of administration is orally through addition of nicotine to the drinking water of animals. The group of Eliakim performed several experiments with oral administration of nicotine to mice or rats. This model was applied to investigate the effect of nicotine on experimental (entero)colitis.[13–15] Also Sykes and Ghia orally administered nicotine to investigate the effect on hapten-induced colitis in rodents.[16,17]

Others injected nicotine subcutaneously or intraperitoneally. Zijlstra and Thomas examined the effect of subcutaneous nicotine administration on the rectal mucosa in rabbits and ferrets.[18,19] Van Dijk et al. combined both above-mentioned methods and treated mice with oral nicotine for 8 days followed by subcutaneous administration.[20] Galitovskiy and colleagues subcutaneously administered nicotine in two murine models of hapten-induced colitis.[21] Snoek et al. gave mice daily intraperitoneal injections of nicotine during the induction of experimental colitis.[22]

Notwithstanding their importance for unveiling some parts of the puzzle on smoking and IBD, there are some concerns about extrapolation of these data to a clinical context. It can be questioned whether oral or parenteral administration is appropriate to mimic (human) smoke inhalation. Especially in the case of oral nicotine administration, a local noxious effect on the intestinal epithelium and mucosal barrier cannot be excluded.

2.2. Other smoke components

A limited number of groups addressed other components of cigarette smoke. The group of Plevy exposed mice to CO to investigate its role in two colitis models.[23,24] Also in the study of Takagi CO inhalation was applied in a murine model of colitis.[25] A group from the University of Hong Kong injected rats intraperitoneally with extracts of either filtered cigarette smoke or used cigarette filters for three consecutive days to examine the effect of various tobacco smoke components on hapten-induced colitis.[26] Finally, the effect of pretreatment with an oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a dioxin compound of cigarette smoke, was studied in experimental colitis.[27,28]

The above-mentioned methods undeniably enhance our understanding about the effect of smoke exposure on the gut. However, extrapolation of their results to the context of human IBD demands some caution. Cigarette smoke is a complex mixture of several thousands of products potentiating or counteracting one another, and administration of only one of these components makes it difficult to draw conclusions about the interaction between smoking and the gut.

Table 1 Administration methods applied in animal smoke models for intestinal research.

Smoke component	Administration route	Duration of exposure	Animal	Ref.
Nicotine	PO, 12.5–250 μg/mL	2–3 weeks	Mouse, rat	13–15
	PO, 5–100 μg/mL	2 weeks	Rat	16
	PO, 20 μg/mL	15 days	Mouse	17
	PO, 10–60 μg/mL	17 days	Rat	29
	SC, 0.5–2 mg/kg/day	2 weeks	Rabbit	19
	SC, 0.3–2 mg/kg/day	10 days	Ferret	18
	SC, 7.5 mg/kg, 2/day	5 days	Mouse	21
	PO, 25–200 μg/mL followed by SC 200 μg/day	8 days PO followed by 2 weeks SC	Mouse	20
	IP, 0.04–0.4 mg/kg	7 days	Mouse	22
CO	Inhalation, 250 ppm	5 days–2 weeks	Mouse	23,24
	Inhalation, 200 ppm	3 days	Mouse	25
TCDD	PO, 5 μg/kg	1 day	Mouse	27
	PO, 15 μg/kg	Day 1 and day 6	Mouse	28
Cigarette smoke	Inhalation, 5–40 puffs/day	1–2 times/day, 17 days	Rat	29
	Inhalation, 2–4% in air	1 h/day, 3–11 days	Rat, mouse	26,31–36
	Inhalation, 1:6 smoke:air ratio	30 min 4/day, 24 weeks	Mouse	39

CO: carbon monoxide, IP: intraperitoneally, PO: per os (orally), SC: subcutaneously, TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin.

2.3. Smoke exposure

Several research groups exposed animals to smoke of burning cigarettes, which we believe approximates best to human smoking.

Galeazzi et al. described a model in which rats inhaled cigarette smoke in a specialized smoking chamber. This allowed a rhythmic smoke inhalation, which is more comparable to human smoking than continuous exposure.[29] The group of Cho applied a smoke model which was first reported in 1996 for the study of the effect of cigarette smoke on gastric ulceration.[30] Rats or mice were placed in a smoking chamber filled with a fixed concentration of cigarette smoke for 1 h daily. This smoke model allowed one to study the effect of smoking on experimental colitis induced by trinitrobenzene sulfonic acid (TNBS),[31–34] by 2,4-dinitrobenzene sulfonic acid (DNBS)[26,35] or by dextran sulfate sodium (DSS).[36,37]

In the above-mentioned models, animals were exposed for only a relatively short time to cigarette smoke (3 to 17 days). In contrast, Verschuere et al. applied a chronic smoke model, created to investigate the role of smoke exposure in chronic airway diseases.[38] Mice were exposed to tobacco smoke four times per day. After 24 weeks, carboxyhaemoglobin in serum of smoke-exposed mice reached a level comparable with that of human smokers (8.70±0.31% versus 0.65± 0.25% in air-exposed animals), suggesting that this model is appropriate for mimicking chronic (active) smoking.[39]

Also these smoke models have limitations. Every group uses its own protocol for smoke:air ratio, duration of exposure and the number of puffs or cigarettes administered per day. Moreover, different types of cigarettes (commercially available versus standardized research cigarettes), with varying amounts of toxic components are used. Standardization of smoke exposure procedures could make comparison of these studies more feasible.

3. Influence of smoke exposure on the normal gut mucosa

Animal studies primarily investigating the effect of cigarette smoke exposure on the normal intestine are relatively scarce.[18–20,39] However, other reports focusing on smoking in intestinal inflammation models also describe their findings on normal gut as secondary end points. Overall, the literature on the impact of smoke exposure on gut histology and physiology presents conflicting results. In some cases, the differences between reports can be explained by dissimilarities in the methods of smoke simulation. In other cases, a dual effect on small bowel versus colon can account for divergence between reports (Tables 2 and 3).

3.1. Inflammatory cells and mediators

A first point of interest is the effect of smoking on inflammatory cells and mediators. There is a consensus that smoke exposure and nicotine administration do not cause macroscopical or histological damage nor inflammation in the gut.[13,14,19,29,31] Furthermore, most authors could not demonstrate an impact on colonic myeloperoxidase (MPO) activity, an index of neutrophil infiltration.[29,31,40] Only one report described a significant, albeit modest, increase of colonic MPO activity after smoke exposure.[32] Also CO inhalation did not cause colonic damage nor changes in MPO activity.[25]

Verschuere et al. reported a recruitment of various immune cell types into Peyer's patches, such as dendritic cells (DCs), CD4+ T-cells, CD8+ T-cells and regulatory T-cells (Tregs). However, this was not associated with tissue damage. The hypothesis is that tolerogenic cell populations (Tregs and CD11b+ DCs) and inflammatory cell types (effector T-cells) reach a new equilibrium without inducing inflammation. Furthermore, Peyer's patch chemokines CCL9 and CCL20, involved in chemotaxis of immune cells, showed an increase in smoke-exposed mice.[39]

Cigarette smoke nor TCDD had any effect on intestinal IgA production in healthy gut.[28,39] Relatively few reports are available on the effect of cigarette smoke or its components on cytokine levels in the gut. Eliakim described a dual effect on cytokines, with a decrease of colonic interleukin (IL)-2 but an elevation of IL-6 and a decline of IL-10 in the jejunum following nicotine administration.[40] The diminished expression of IL-10 following smoke exposure was confirmed in ileal Peyer's patches, but inflammatory cytokine levels in Peyer's patches were unaltered.[39] Van Dijk reported a significant decrease of the inflammatory cytokines IL-1β and

Table 2 Effect of smoke or nicotine exposure on small bowel in normal conditions.

Parameter	Effect	CS constituent	Animal	Ref.
Macroscopy	No difference	Nicotine	Mouse, rat	13,14
Histology	No difference	Nicotine	Mouse, rat	13,14
Inflammatory cells	Recruitment of T-cells and DC	CS	Mouse	39
Cytokine levels	IL-6↗, IL-10↘	Nicotine	Rat	40
	IL-1β =, TNF-α =, IFN-γ =, IL-6 =, TGF-β =, IL-10↘	CS	Mouse	39
Chemokine levels	CCL9↗, CCL20↗	CS	Mouse	39
PGE2	↘	Nicotine	Rat	14
Apoptosis	↗ in FAE, = in VE	CS	Mouse	39
Mucus constituents	ITF =, MUC2 =	Nicotine	Mouse	13
Luminal IgA	=	CS	Mouse	39
ROM production	iNOS activity ↗	Nicotine	Rat	14

CS: cigarette smoke, DC: dendritic cells, FAE: follicle-associated epithelium, IL: interleukin, iNOS: inducible nitric oxide synthase, ITF: intestinal trefoil factor, PGE2: prostglandin E2, ROM: reactive oxygen metabolite, VE: villous epithelium.

Table 3 Effect of smoke or nicotine exposure on colon in normal conditions.

Parameter	Effect	CS constituent	Animal	Ref.
Macroscopy	No difference	CS, nicotine, CO	Mouse, rat, rabbit	13,14,19,25,29,31
Histology	No difference	CS, nicotine	Mouse, rat, rabbit	13,14,19,29,31
MPO activity	No difference	CS, nicotine, CO	Rat, mouse	25,29,31,40
	↗	CS	Rat	32
Cytokine levels	Il-2↘, IL-6 =, IL-10 =	Nicotine	Rat	40
	IL-1β↘, TNF-α↘	Nicotine	Mouse	20
	TNF-α =	CO	Mouse	25
PGE2	=	Nicotine	Rat, rabbit, mouse, ferret	14,18,20,40
	↗	CS	Mouse	37
Apoptosis	=	CS	Mouse	36,37
Mucus constituents	ITF↗, MUC2 =	Nicotine	Mouse, rabbit	13,19
Luminal IgA	=	TCDD	Mouse	28
ROM production	ROMs =, XA activity =, iNOS activity =	CS, nicotine	Rat	14,31,32
	SOD activity ↘, GSH =	CS	Rat	31,32

CO: carbon monoxide, CS: cigarette smoke, DC: dendritic cells, GSH: glutathione, IL: interleukin, iNOS inducible nitric oxide synthase, ITF: intestinal trefoil factor, MPO: myeloperoxidase, PGE2: prostglandin E2, ROM: reactive oxygen metabolite, SOD: superoxide dismutase, TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin, XA: xanthine oxidase.

tumor necrosis factor (TNF) α in the colonic mucosa of mice after nicotine treatment.[20] Overall, these studies suggest that smoke and nicotine exposure on one hand cause a decrease of the anti-inflammatory cytokine IL-10 in the small bowel, but on the other hand attenuate inflammatory cytokine levels in the colon. CO was not found to influence colonic TNF-α levels. However, its possible impact on other inflammatory and immunosuppressive cytokines was not studied.[25]

Data on the impact on mucosal eicosanoids are inconsistent and strongly depend on the type of smoke model used. Nicotine exposure induced a significant decrease in jejunal prostaglandin (PG) E2 generation but had no influence on colonic PGE2.[14,19,40] Data on the influence on rectal eicosanoid levels are conflicting. Zijlstra described a significant decrease of various eicosanoids after nicotine treatment. This possibly implicates a decreased protective capacity in the rectum against inflammatory stimuli.[19] However, more recent reports of his group could not demonstrate an effect of nicotine on rectal prostaglandins.[18,20] In contrast with these data from nicotine models, increased colonic concentrations of PGE2 and leukotriene B_4 (LTB_4) were observed following smoke exposure.[31,32,37] This might be explained by the interaction of dioxins in cigarette smoke with aryl hydrocarbon receptors in the colon, inducing PGE2 production.[41,42]

3.2. Mucosal barrier and epithelial lining

Another focus is the impact of smoking on intestinal epithelium and the mucosal barrier. Two groups investigated the effect of cigarette smoke on intestinal epithelium. Verschuere investigated the follicle-associated epithelium (FAE) covering murine Peyer's patches, because this is considered to be the first affected region in early CD.[43,44] Following smoke exposure, an increased apoptotic index in the FAE was observed. In contrast, the neighboring ileal villous epithelium did not show induction of apoptosis. This suggests that the FAE is more vulnerable to smoke-induced apoptosis than the absorptive epithelium.[39] Liu found no effect of smoke exposure on apoptosis in the colonic mucosa nor the colonic epithelium, confirming that absorptive epithelium is more resistant to the noxious effects of smoke exposure.[36,37]

Only two studies addressed the influence of nicotine and smoke exposure on protective peptides and the mucus layer. Nicotine treatment did not affect jejunal or colonic expression of the neuropeptide somatostatin or the mucin MUC2. The effect on other mucus layer constituents was dual; while nicotine induced expression of colonic intestinal trefoil factor (ITF), no changes were seen in jejunal ITF.[13] Nicotine affected the thickness of the adherent mucus layer in the rectum, with a significant reduction of the mucus layer in the low dose group but an increase in mucus layer in the high dose group.[19]

3.3. Oxidative stress

Intestinal damage and inflammation are associated with oxidative stress, caused by reactive oxygen metabolites (ROMs) such as superoxide and N-chloramines. ROMs are produced by several biochemical pathways involving many enzymes, such as xanthine oxidase (XO) and inducible nitric oxide synthase (iNOS). On the other hand, antioxidant systems, including superoxide dismutase (SOD) and low-molecular-weight antioxidant molecules, such as reduced glutathione (GSH), function as scavengers for free radicals and are cytoprotective against oxidative damage (Fig. 1). In homeostatic conditions, a delicate balance exists between oxidants and anti-oxidants.

Cigarette smoke can interfere with this balance through several mechanisms. Next to mediating the pathways that generate endogenous ROMs, cigarette smoke also contains a high concentration of ROMs itself, including nitric oxide and superoxide in the gas-phase and semiquinone-radicals in the tar-phase. Furthermore, it contains metal ions such as iron, which allow the transformation of hydrogen peroxide to the highly reactive hydroxyl radical.[45]

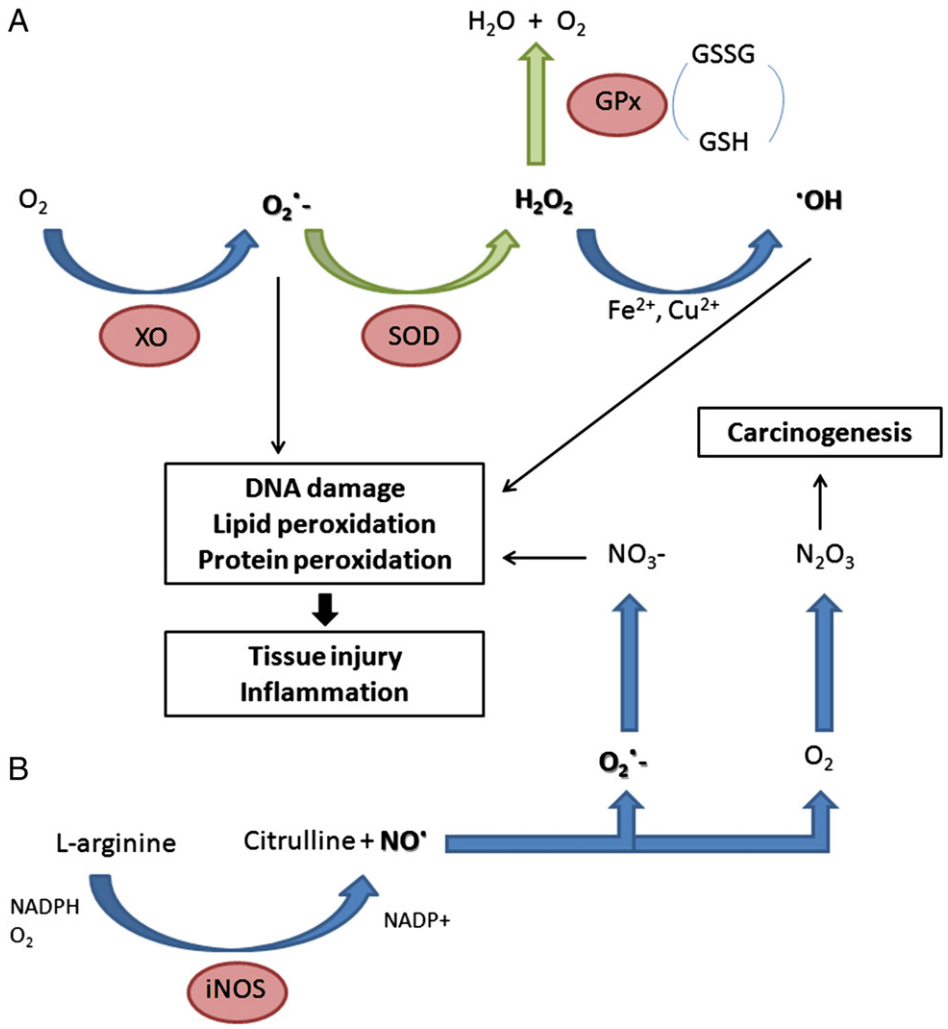

Figure 1 Mechanisms of oxidative damage. Panel A shows the generation of reactive oxygen metabolites (ROMs). These metabolites can be formed by an enzymatic reaction out of oxygen. However, several ROMS are also present in cigarette smoke (bold). Panel B shows the production of reactive nitrogen metabolites. These are normally formed as a side product of the transformation of L-arginine into citrulline by NOS. But as is the case with ROMs, several nitrogen reactive metabolites are components of cigarette smoke. The formation of highly reactive metabolites including superoxide anion, the hydroxyl radical and NO_3^- result in damage to DNA, lipids and proteins causing tissue damage. The green arrows indicate protective anti-oxidant pathways, which decrease oxidative stress. GSH: gluthatione, GSSG: oxidized gluthatione, GPx: gluthatione peroxidase, iNOS: inducible nitric oxide synthase, SOD: superoxide dismutase, XO: xanthine oxidase.

Cigarette smoke or nicotine exposure did not induce changes in colonic ROM production, nor in colonic XA activity or iNOS activity. However, jejunal iNOS activity increased after nicotine administration, possibly pointing towards an increased generation of nitric oxide and an elevated oxidative stress in the small intestine.[14,31,32] GSH levels in the colon were not altered by smoke exposure, but colonic SOD activity decreased significantly.[31,32] This can implicate a decreased radical-scavenging ability of the colon after smoke exposure, making the gut more susceptible for oxidative damage to cells and organelles.

4. The effect of smoking on gut inflammation

A variety of genetical and chemically-induced colitis models were used to study the impact of smoke or nicotine exposure on gut inflammation (Table 4). Both models mimicking CD and UC were studied.

4.1. TNBS-induced colitis

A first model is the administration of a TNBS enema, resulting in a colitis type which shares more similarities with human CD than with UC.[21] This model was used repeatedly by the group of Cho, in combination with passive smoke exposure. They described a potentiating effect of cigarette smoke on TNBS-induced colitis, with a dose-dependent increase in macroscopical lesions, MPO activity and LTB4 and TNF-α concentrations in the distal colon. Microscopically, smoke-exposed rats showed more tissue damage and inflammation in comparison to controls.[31–34] A possible mechanistic explanation of this effect involves increased oxidative

Table 4 Influence of cigarette smoke and cigarette smoke components on experimental (entero)colitis.

Inflammation model	Smoke constituent	Effect on intestinal inflammation	Ref.
TNBS colitis	Cigarette smoke	Potentiating effect	31–34
	SC nicotine	Potentiating effect	21
	PO nicotine	Low dose: attenuating effect High dose: potentiating effect or no effect	15,16
	CO	Attenuating effect	25
	PO TCDD	Attenuating effect	28
Iodoacetamide	PO nicotine	Jejunitis: potentiating effect Colitis: attenuating effect	14
IL-10−/− mice	PO nicotine	Jejunitis: potentiating effect Colitis: attenuating effect	13
	CO	Attenuating effect	23
DNBS colitis	Cigarette smoke	Attenuating effect Potentiating effect	26,35 29
	SC nicotine	Attenuating effect	26
	PO nicotine	Potentiating effect	29
Oxazolone colitis	SC nicotine	Attenuating effect	21
DSS colitis	Cigarette smoke	No effect	36
	PO nicotine	Attenuating effect	17
	IP nicotine	No effect	22
	PO TCDD	Attenuating effect	28
TCRα−/− mice	CO	Attenuating effect	24

CO: carbon monoxide, DNBS: 2,4-dinitrobenzene sulfonic acid, DSS: dextran sulfate sodium, IP: intraperitoneally, PO: per os, SC: subcutaneous, TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin, TNBS: trinitrobezene sulfonic acid.

stress due to smoke exposure. The effect of TNBS on iNOS activity, on GSH concentration and on ROM production was aggravated by cigarette smoke. In contrast, the TNBS-induced rise in SOD and XO activity was attenuated by smoke exposure, decreasing the anti-oxidant capacities.[31,32]

On the contrary, the other findings in this model appeared to have rather a protective function than a damaging role in intestinal inflammation. Smoke exposure had no effect on TNBS-induced colonic PG synthesis, or on expression of *cyclooxygenase* (*COX*)-1, but potentiated expression of *COX*-2. COX-2 is involved in the synthesis of PGs and exerts a protective effect on mucosal damage in colitis.[33] Furthermore, they demonstrated that cigarette smoke causes a TNBS-independent increase in colonic expression of the α7 subunit of the nicotinic acetylcholine receptor (nAChR), which is an essential regulator of inflammation by inhibiting the release of TNF by macrophages.[34,46]

Galitovskiy and colleagues confirmed the aggravating effect on TNBS colitis by nicotine administration. They reported a significantly higher DAI (disease activity index) score and more severe colonic inflammation, as assessed both macroscopically and microscopically. Moreover, nicotine caused a further increase of CD4+ T-cells in the lamina propria of mice treated with TNBS. The Treg population remained unaltered, whereas the Th17 T-cells additionally increased after nicotine administration. Finally, nicotine potentiated the TNBS-induced elevation of the inflammatory cytokines IL-23 and IFN-γ.[21]

Also other groups used the TNBS model of experimental colitis, but their findings conflicted with the data from Guo and Galitovskiy. Both Eliakim and Sykes reported a decrease in the macroscopical lesion area, histological damage and MPO activity in nicotine-treated TNBS colitis. The potentiating effect on LTB4 levels and the lack of impact on PGE2 generation was confirmed.[15,16] However, the observed effect of nicotine on oxidative stress differed greatly from the findings of Guo. Eliakim found no effect of nicotine on TNBS-induced colonic NOS activity.[15] Sykes et al. demonstrated that nicotine attenuated the increase of iNOS and, to a lesser degree, of TNF-α.[16] Interestingly, both authors reported that the attenuating effect of nicotine on TNBS-induced inflammation and damage was more evident after administration of a modest dose, while a high nicotine dose had a smaller effect or even aggravated the induced lesions.

CO inhibited TNBS colitis, as assessed both macroscopically and histologically. Furthermore, MPO activity and TNF-α levels attenuated after CO inhalation.[25]

Finally, an amelioration of TNBS colitis was described after administration of the dioxin TCDD, both clinically and histologically. The effect of TCDD was mediated by aryl hydrocarbon receptor activation on immune cells, causing a suppression of the inflammatory colonic cytokines IL-6, TNF-α, IFN-γ and IL-12. In contrast, the colonic expression of IL-10 and IL-17 remained unaltered. TCDD also generated Tregs in TNBS colitis. So both a decrease in inflammatory mediators and an increase in Tregs can account for the amelioration of colonic inflammation in this model. Furthermore, TCDD was able to modulate humoral gut immune responses in TNBS colitis, including an increase in IgA.[28]

Summarized, literature on the effect of cigarette smoke and its components on TNBS-induced colitis revealed conflicting results. Two studies using nicotine administration reported a dose-dependent effect on intestinal inflammation, so the inconsistent data can be explained to a certain extent by differences in nicotine dosage and means of

administration. Furthermore, it appears that different smoke components affect TNBS colitis in divergent ways, leading to opposing results. This might explain the conflicting epidemiological data on human CD colitis, with different studies describing divergent outcomes of smoking and nicotine therapy on the disease course of CD colitis.[8,47–49]

4.2. Iodoacetamide-induced enterocolitis

A second animal model of chemical enterocolitis is the use of iodoacetamide, inducing lesions mimicking CD. This model not only investigated the colon but also the jejunum. Nicotine had diverging effects on inflammation in the small bowel and the colon. Jejunitis aggravated, both macroscopically and microscopically. Furthermore, jejunal NOS activity increased and PGE2 decreased, suggesting a role for oxidative damage and decreased mucosal protection by PG in the damaging effect of nicotine on small intestinal inflammation. In contrast, colonic inflammation was attenuated by nicotine, as assessed by lesion area and histological score. Colonic NOS activity remained unchanged.[14]

4.3. Enterocolitis in IL-10−/− mice

The next model discussed is a genetical model, IL-10 deficient (IL-10−/−) mice, which spontaneously develop chronic enterocolitis with features similar to CD. After nicotine administration, jejunitis deteriorated significantly, both grossly and histologically, with more extensive and multifocal lesions. In contrast, colitis in IL-10−/− mice ameliorated, as could be appreciated both macro- and microscopically. The protective effect on colitis can be modulated through increased production of protective peptides and mucus components. Colonic expression of *ITF* and *somatostatin* increased significantly after nicotine administration, whereas jejunal concentrations remained unchanged. Expression of *MUC2* was unaltered in both jejunum and colon.[13]

A second group investigated the effect of CO on colitis in IL-10−/− mice. Histological improvement was observed, together with decreased colonic levels of IL-12 p40 and TNF. Unfortunately, the effect on the small bowel was not studied.[23]

4.4. DNBS-induced colitis

A frequently used colitis model entails the administration of a DNBS enema to induce a chemical colitis mimicking UC rather than CD. Two groups used this model, but again with conflicting results. Galeazzi investigated the impact of both inhaled cigarette smoke and oral nicotine, and reported a deterioration of DNBS colitis (both macroscopically and microscopically) and a potentiating effect on MPO activity after smoke exposure. Nicotine administered in high doses led to similar results.[29]

In contrast, Ko described an attenuation of DNBS colitis by smoke inhalation as well as subcutaneously administered nicotine. Furthermore, smoke and nicotine exposure downregulated the DNBS-induced increase of LTB4 levels and concentrations of TNF-α, IL-1β and IL-6. Also induction of ROM production and iNOS activity in DNBS colitis was attenuated.[26,35]

As both groups investigated smoke exposure as well as nicotine treatment, it is unclear what causes the discrepancies between these studies.

4.5. Oxazolone-induced colitis

One study examined the effect of nicotine on oxazolone colitis, a murine model mimicking UC colitis. Nicotine-treated animals showed a decreased severity of colitis, as evaluated by the DAI score and macroscopical and histological examination of the colon. Furthermore, nicotine further increased the percentage of CD4+ T-cells in the colonic lamina propria. When examining different lamina propria T-cell subsets, a significant rise in Tregs was observed, whereas Th17 cells tended to decrease following nicotine administration.

The authors hypothesized that the observed nicotine-induced changes are mediated by nAChRs, causing alterations in T-cell development and function and modifying cytokine production. Indeed, oxazolone-exposed T-cells had increased levels of α7 nAChRs. Consistent with this, nicotine lost his attenuating effect on oxazolone colitis in α7 knockout mice, indicating that the α7 nAChR plays a crucial role in the activity of nicotine.[21]

4.6. DSS-induced colitis

Snoek and colleagues used the acute model of DSS colitis to study the effect of nicotine on an UC type of colitis. Nicotine did not induce clinical improvement of colitis, as assessed by clinical parameters and histopathological score. Although colonic levels of the inflammatory cytokines IL-6 and IL-17 were significantly reduced following nicotine administration, no alterations in TNF levels were observed.[22]

The DSS colitis model was also applied by Liu et al. Their results were comparable with the reported data of Snoek; nor assessment of edema, nor pathological scoring of the severity of inflammation could reveal differences between the smoke-exposed and the control group.[36]

Also Ghia examined the effect of nicotine on DSS colitis. However, the results of this study are in sharp contrast with the two afore-mentioned reports. Nicotine was found to decrease both macroscopical and microscopical damage, as well as MPO activity. Furthermore, a decrease in TNF and IL-1β was described, whereas IL-6 levels remained unaltered.[17] Differences in administration ways may account for the contradictory effects described in these studies, with orally administered nicotine exerting not only a systemic effect, but also a local effect on the mucosal barrier.

Pretreatment with the dioxin TCDD inhibited DSS-induced colitis, as assessed by decreased weight loss, reduced inflammation and lower levels of heme oxygenase-1 (HO-1), MPO and TNF-α. Its effect is likely to be mediated through induction of PGE2 production.[27] However, also the generation of Tregs can partly account for the immunosuppressive effect of TCDD.[28]

4.7. Colitis in TCRα−/− mice

A last model mimicking UC is a genetical model in which the gene of TCRα is disrupted. These mice develop spontaneously colitis with a Th2 signature. When these mice were

exposed to CO, a significant amelioration in body weight and inflammation was observed. This was associated with a decreased production of inflammatory cytokines, such as IL-1β, IL-4, TNF and IL-17 and increased production of IL-10 by colonic macrophages.[24]

Overall, CO appears to have a positive effect on cytokine levels in every colitis model investigated. This effect is at least partly HO-1 mediated.

5. Smoke exposure and inflammation-induced carcinogenesis

An important pathway in the development of colorectal carcinoma is inflammation-associated neoplasia. IBD, and especially UC, is associated with an increased risk for development of colorectal cancer. A correlation exists between the extent and longevity of inflammation and the risk of cancer.[50,51] Also smoking is an independent risk factor for colorectal neoplasia.[11] However, smoking is considered as a protective factor in UC as well, ameliorating the severity and extent of colitis.[9] Several authors suggested that smoking protects against cancer in UC patients, although no consensus exists on the role of cigarette smoke in inflammation-associated carcinoma in the colon.[52,53]

Liu et al. investigated whether smoke exposure counteracts the detrimental effect of colonic inflammation on carcinogenesis or, conversely, enhances inflammation-associated neoplasia. Epithelial proliferation index did not increase immediately after smoke exposure but was significantly higher 1 month later, pointing to a possible carcinogenic effect of tobacco smoke in the pathogenesis of colorectal neoplasia. Smoke exposure increased dose-dependently the incidence of both dysplasia and carcinoma one month after DSS colitis. Also the number of adenomatous polyps increased after cigarette smoke exposure. The authors suggest that although cigarette smoke alone was not able to cause dysplasia, it provides an abundant source of carcinogens. This increases the risk for DNA repair errors during the rapid cell turnover in the context of intestinal inflammation, resulting in a higher number of mutant cells and replication errors in mucosal regeneration.[36,37]

6. Translation of data from animal studies to a clinical context

Valuable information can be deduced from the data discussed above regarding the effect of smoke and its constituents on both normal gut function and intestinal inflammation.

In physiological conditions, cigarette smoke does not cause macroscopical or microscopical damage to the gut. However, smoking appears to make the small intestine more susceptible to events that trigger inflammation. The small bowel becomes less tolerogenic with a decrease of IL-10[39,40] and a recruitment of inflammatory cells towards the Peyer's patches.[39] Furthermore, the concentration of PGE2, which protects the gut against mucosal damage, decreases and a higher iNOS activity is observed, leading to increased oxidative stress for the intestinal epithelium.[14] In the colon, the impact of smoke exposure is less clear. The colonic cytokine profile following smoke exposure is more favorable, with a decrease in several inflammatory cytokines.[20,40] The increased expression of ITF is also a protective factor.[13] However, the observed decrease in colonic SOD activity is rather harmful, as this diminishes the scavenging capacity toward ROMs.[31,32] In summary, the effect of smoke and its constituents on the colon appears ambiguous, whereas on the small bowel it is deleterious.

The interaction of tobacco smoke and its constituents with experimental (entero)colitis is even more liable to dual interpretation. Depending on the model for experimental inflammation applied, an aggravating or ameliorating effect is seen. Especially in TNBS- and DNBS-induced colitis, the data from different groups are inconsistent. Various explanations can be given for the divergent results discussed in this review. First of all, in the different models a variety of species were used. It is not established yet whether these species react similarly towards smoke exposure. Secondly, smoke administration methods differ greatly, ranging from inhalation of cigarette smoke and CO to orally or subcutaneously administered nicotine, applied in diverse doses and during diverging time intervals. Thirdly, some authors focus on the small bowel, while others investigate the colon or both organs. Finally, different models of experimental (entero)colitis were used to study the effect of cigarette smoke on intestinal inflammation. Every IBD model has its own strengths and weaknesses, and approximates more to human CD or UC. In most studies, the effect of smoke exposure on acute inflammation was considered, whereas the IL-10−/− model and repeated DSS administration offer a model for chronic relapsing inflammation, more resembling human IBD. To make a comparison of the data from different studies more feasible, standardization of smoke models in gastrointestinal research is imperative. No data are available on the effect of smoking cessation on colonic inflammation. This would be interesting, given the increased risk to develop UC after smoking cessation in humans.

The best studied cigarette smoke component is undeniably nicotine. Nicotine is considered as an important mediator of intestinal inflammation with immunomodulating capacities. Therapeutic use of nicotine is extensively studied in human UC. However, conflicting data on the efficacy and safety of nicotine treatment impede its implementation in standard UC therapy.[54,55] The mechanism through which nicotine exerts its immunosuppressive effect, is most likely the 'cholinergic anti-inflammatory pathway', a concept first introduced by Tracey.[56] This pathway modulates systemic inflammatory responses through efferent vagal nerve signaling. Hereby neurotransmitters, such as nicotine and acetylcholine, interact with nAChRs on immune cells in peripheral organs.[57] It was shown that nicotine signaling inhibits TNF release by human macrophages after triggering with endotoxin, and this is modulated by interaction with the α7 nAChR on macrophages.[46,56] Cigarette smoke exposure was demonstrated to increase colonic expression of the α7 nAChR, and this might account for the protective effect of smoke or nicotine exposure on experimental colitis, down-regulating TNF-induced inflammation.[34] Interestingly, a recent publication illustrated a dual effect of nicotine in two different models of experimental colitis. This was due to a differential expression of α7 nAChRs by colonic T-cells. The Th2 cytokine milieu in oxazolone-induced colitis facilitated expression of α7 nAChR, whereas the Th1-type

cytokines in TNBS colitis abolished α7 nAChR expression. Interaction of nicotine with this receptor skewed the Treg/Th17 balance towards the immunosuppressive Tregs, resulting in attenuation of colonic inflammation and improvement of the clinical condition. The results of this elegant study can help to understand the opposing effects of smoking on UC and CD.[21]

However, although nicotine unquestionably plays a major role in the effect of smoking on the gut, there is no evidence that it is the sole active mediator in cigarette smoke. To some extent, nicotine and smoke exert the same effect on the gut. However, other tobacco components also intervene, augmenting or suppressing the influence of nicotine on gut immunology and physiology. For example, the aryl hydrocarbon receptor pathway is implicated in the preventive effect of smoking on experimental colitis and UC. Administration of TCDD, a dioxin component of cigarette smoke, results in attenuation of colitis in several models of experimental colitis (TNBS, DSS). Hypotheses on how TCDD exerts its effect include induction of colonic PGE2,[27] down-regulation of inflammatory cytokines,[28,58] and generation of Tregs.[28] Therefore, we believe that no premature conclusions should be drawn from animal studies administrating a sole smoke constituent.

Notwithstanding the differences between human active smoking and the above-mentioned animal models mimicking smoking, some interesting conclusions can be drawn for the impact of smoking on human IBD. In the past, several hypotheses were formulated about the dual effect of smoking on CD and UC. Some authors assumed that this difference was due to disease type (Th1 response in CD versus Th2 response in UC), causing a distinct cytokine milieu in which the effects of the different smoke components can be modified.[21] Others proposed that genetical predisposition, resulting in divergent metabolism of smoke constituents, played a role making an individual more vulnerable to the immunosuppressive or immunogenic effect of individual smoke components.[59,60] A last hypothesis is that not the disease type, but the disease location determines the effect of smoking. Small bowel disease, like Crohn's ileitis,

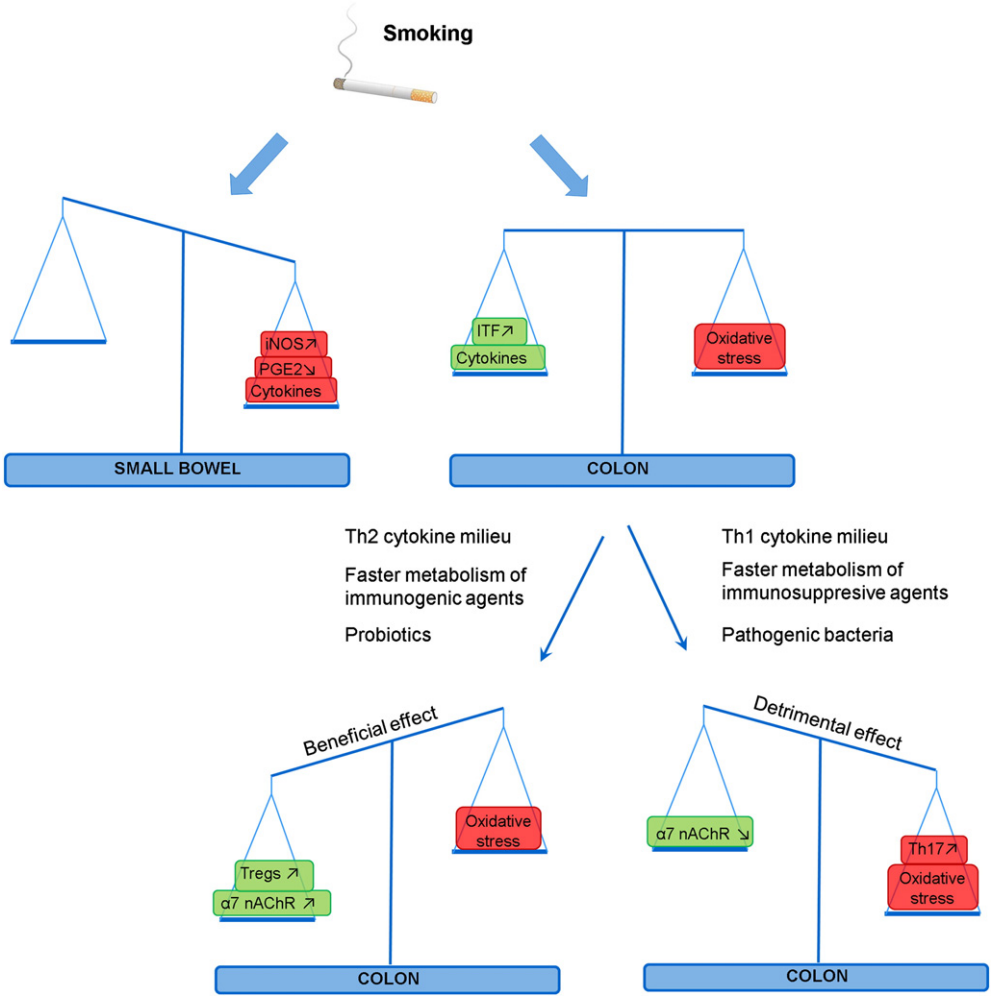

Figure 2 Divergent effect of smoking on the small bowel and the colon. Evidence from animal models shows that smoke exposure has a detrimental effect on the small bowel immunology, predisposing it towards increased vulnerability to inflammatory stimuli.[13,14,39,40] For the colon, more ambiguous effects are described in the different models.[13,20,31,32,40] Other factors, such as the intestinal commensal flora, viral or bacterial pathogens, and genetic predisposition manipulating the metabolism of various smoke constituents will determine whether beneficial or rather unfavorable effects will take over.

is negatively influenced by cigarette smoke, whereas colonic inflammation often ameliorates under smoke exposure. In favor of this hypothesis is that literature describes a worse outcome for Crohn's ileitis than for Crohn's colitis, although also Crohn's colitis is negatively influenced by active smoking.[8,48] Indeed, when examining the current available data on smoke animal models in IBD research, the divergent effect of cigarette smoke on small bowel and colon is confirmed. Whereas the small bowel appears to be driven towards susceptibility for inflammatory stimuli and increased risk for developing enteritis, the situation for the colon is less clear. Cigarette smoke constituents exert both immunosuppressive and immunogenic effects on the colon, leading to respectively an attenuation or aggravation of colitis. We hypothesize that the effect of smoking on colitis depends on other factors, such as genetical predisposition, cytokine environment and environmental factors (Fig. 2). Further research will need to elucidate the conditions in which cigarette smoke exerts a beneficial or rather an injurious effect on colonic inflammation.

7. Conclusion

What lessons can we learn from these animal models on the impact of smoking on human gut in physiological and pathological conditions? Smoking appears to have a divergent effect on small bowel and colon, leading to a less tolerogenic phenotype in the small bowel and both beneficial and damaging alterations in the colon. This dual effect on the colon is also reflected in IBD models, giving conflicting results about the impact of cigarette smoke on colitis, whereas enteritis is negatively influenced by smoke exposure in every animal model tested. Nicotine plays a modulating role in intestinal inflammation through interaction with the α7 nAChR on intestinal immune cells. However, the impact of other smoke compounds should not be neglected. More and better standardized research will help to elucidate the remaining questions on the exact role of smoking on the gut in health and disease.

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