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Biomedicine & Pharmacotherapy 129 (2020) 110404

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Review

Clinical implications of nicotine as an antimicrobial agent and immune modulator

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ARTICLE INFO

Keywords:
Nicotine
Anti-microbial activity
Immune response
Microbiome

ABSTRACT

Nicotine is perhaps the most important and potent, pharmacologically active substance in tobacco products. This commentary examines the possible effects that nicotine has on microbial viability and also on the host's immune system as it responds to the indigenous microflora (the microbiome) due to nicotine-induced changes to the indigenous microbial environment and any associated antigenic stimulation / immunization that may occur. To our knowledge, the analysis of such profound microbiologic changes attributable to a tobacco-related product, such as nicotine, has not been fully explored in the context of its consequences on the viability of the microbiome/microbiota and on some of the host's basic physiologic processes, such as the immune response, and its possible association on the induction and persistence of certain immunologically related diseases. Future studies should be aimed at uncovering the molecular mechanisms involved in such interactions, especially in the context of manipulating them for therapeutic purposes.

1. Introduction

Both the upper and lower regions of the alimentary canal (oropharyngeal cavity and gastrointestinal [g.i.] tract) are typically sites of almost constant and sometimes intense physiologically-related activity primarily related to the processing of liquid or solid food products. In these locations there is a complex and densely populated mixture of usually harmless yet essential microorganisms (the microbiome), a few potential pathogens that are capable of being inhaled, ingested or aspirated, and many complex macromolecules capable of eliciting, as well as regulating, various immune responses. The local mucosal immune system has developed mechanisms for readily ignoring the "harmless" resident microflora and most of the inhaled/ingested substances (potential antigens/superantigens), while generating protective responses, such as locally produced immunoglobulins, especially IgA, to a wide range of pathogens. IgA also helps preserve the composition of the gut microbiome [1], but it has also been shown that, under certain circumstances, it does not change significantly in the absence of secretory IgA. Another example of an extensive immune network that could be affected by such events would be the gut-associated lymphoid tissue (GALT) which is distributed throughout the gastrointestinal system. In this context, the potential of nicotine to interfere with the growth of various microorganisms and/or convert them into a more potent antigenic stimulus could be a significant concern. Such a result would have broad range implications, since a large segment of the human population uses nicotine-containing tobacco products or nicotine alone for therapeutic purposes (primarily for withdrawal relief) [2,3].

2. Discussion

2.1. Effect of nicotine on various key metabolic processes

While it is now reasonably well established that the use of tobacco is a major health hazard [4,5], the World Health Organization estimates that about one billion of the world's population still continue to use various tobacco products including the now more popular smokeless varieties despite the numerous deleterious effects they have on the human body [6]. Lung cancer is caused primarily by smoking, and oral cancers are strongly linked to those who use chewing tobacco or snuff. Much of the damage caused by tobacco is usually attributed to the direct toxicity or carcinogenicity of some of the many chemicals present in tobacco products, making it difficult to distinguish which ones cause the most harm with nicotine being considered to be the most pharmacologically active [6]. It is also possible that some of these same

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https://doi.org/10.1016/j.biopha.2020.110404
Received 16 March 2020; Received in revised form 21 May 2020; Accepted 13 June 2020
Available online 27 June 2020

compounds present in tobacco may indirectly have deleterious effects on humans by interfering with the normal functioning of the host defense (surveillance) system leading to increased susceptibility to neoplastic diseases and/or by lowering resistance to certain infections including, more recently and now receiving considerable scrutiny, the coronavirus, COVID-19. Some of these undesirable byproducts might possibly be due to the interaction of nicotine with the indigenous microflora [7,8] and with various host defense mechanisms affecting both humoral and cellular immune responses involved in protecting against invading pathogens. Indeed, several studies [9–11] have shown that tobacco smoke, smokeless tobacco and nicotine alone can be detrimental to certain components of the immune system of humans and experimentally exposed animals. It is noteworthy, however, in this regard, that although it is somewhat accepted that tobacco products have an adverse effect on humoral and cell-mediated immunity, neither the extent of this impairment nor of its mechanisms have been clearly elucidated. Many of these immunologic changes can occur locally, for example, in the lung and g.i. tract primarily affecting GALT [12] as well as systemically [5,13] and they are generally reversible with cessation of tobacco exposure. It is not known, however, whether long-term use of tobacco can cause permanent or selective damage to the immune system or to some of its components and whether such events are associated with concomitant effects on the microbiome.

In light of recent developments associated with maternal-fetal interactions [14] during pregnancy and the early post-partum period, it is interesting to speculate on whether the composition of the microbiome in early childhood would vary in a smokeless home environment relative to one not free of a family of smokers as a result of exposure to second-hand smoke, along with knowing when does microbial colonization become fully entrenched in the human body or changes during the course of a person's lifetime. Also in this context, the collective role of other significant lifestyle factors such as dietary and eating patterns and certain related underlying conditions or co-morbidities (for example, asthma, diabetes and obesity) [15] in affecting the make-up of especially the commensal gut microbes and their normal function, along with the innate and adaptive immune response pattern in the gut, should be considered. Perhaps equally intriguing is the possible beneficial role that one of the key components of tobacco, nicotine, may have towards interfering with unwanted inflammatory responses [16] such as those that may occur in the gastrointestinal tract with its vast and complex array of indigenous bacteria. These consist primarily of gram-negative bacilli (the so-called enterobacteriaceae) and a select group of gram-positive cocci and bacilli. Collectively, these include aerobes, facultative and strict anaerobes, and a small group of spore-forming organisms. Also, in this context, other factors need to be considered including the recent finding [17] that the sleeping aid and therapeutic agent, melatonin, may have broad ranging effects on GALT and the metabolism of the gut microbiome. Moreover, additional studies [reviewed in ref. 18] have shown how the microbes residing in the g.i. tract may affect the efficacy of some of the more novel immunotherapeutic interventions for treating certain types of cancers especially those designed to block CTLA-4 and PD-1 immune checkpoint molecules which would then enable cytolytic CD8 + T cells to kill neoplastic cells.

2.2. Interaction of nicotine with various microbes and possible modulation of immune function

Apart from their possible deleterious effects on the immune system especially in the context of increasing susceptibility to infections [19], little is known on whether tobacco-related products could have direct detrimental activity against microorganisms, including those comprising the microbiome [20]. This possibility would be especially noteworthy for certain parts of the body, such as the oral cavity, where exposure to tobacco-related products occurs most commonly and would be most intense [21]. Accordingly, any prolonged antimicrobial effects occurring here could alter the balance of, and/or the indirect protective effects afforded by, the oral microflora which could lead to colonization or overgrowth of unwanted pathogens in the oropharynx. It also may have some bearing on certain periodontal diseases such as periodontitis (known to be initiated by bacteria). Such ecological alterations might also affect local immune response patterns and/or susceptibility to certain pathogens, especially with any concurrent physical damage to the oral mucosal architecture [21]. To our knowledge, the development of such profound microbiologic changes attributable to tobacco-related products has not been fully explored and experimental studies should be designed to analyze this issue.

A more recent development, designed for dealing with the health consequences of tobacco product use, has involved the implementation of nicotine replacement therapy such as over-the-counter gums [3], sublingual tablets/lozenges [3] and transdermally applied patches [22] for those who need aid in attempting to cease smoking and to reduce withdrawal symptoms. Since nicotine is considered to be the most addictive substance [6] in tobacco products, its usage in these alternative forms is being touted as a safe and alternative method for dealing with nicotine withdrawal symptoms as a result of abstaining from smoking or from resorting to using smokeless tobacco. Similar to tobacco in its entirety, limited information (reviewed in ref. #[23]) exists on the manner in which nicotine might interact with microorganisms and with the host's immune system. There is some evidence [9,13] that nicotine can influence certain aspects of humoral and cellular immunity including the lowering of IgA levels in both saliva and intestinal secretions, the inhibition of cytokine production in vitro by peripheral blood and intestinal mononuclear cells, and the induction of certain forms of both T- and B- lymphocyte anergy. Also, along these lines and from a therapeutic perspective, certain clinical studies [16] have revealed an additional partial beneficial use for nicotine: as an alternative treatment modality for inflammatory bowel disease (IBD), specifically ulcerative colitis. Its benefit, however, appears to be limited to treating active colitis and not for maintaining remission [16]. Although its mechanism of action here is not fully known, current evidence [11,24] suggests that nicotine significantly affects cytokine levels, including pro-inflammatory mediators, in the colon. Such cytokines, especially tumor necrosis factor (TNF) play a key role in the development and progression of Crohn's disease [25] and ulcerative colitis [26], the most severe forms of IBD. Over the past several years, they have become the targets for the commercial development and therapeutic use of a unique form of immunotherapy especially for Crohn's disease as well as other highly inflammatory conditions, one of which involves the infusion of a monoclonal antibody directed against TNF [25,27].

It is worth noting that the current belief is that IBD results from the combined effects of changes in the host interactions with the intestinal microbiome, intestinal epithelial dysfunction, aberrant mucosal immune responses, and altered composition of the intestinal commensals [28]. Nucleotide oligomerization binding domain 2 (NOD2) is a gene associated with Crohn's disease. It encodes an intracellular protein that binds to bacterial peptidoglycans [28]. Other genes such as autophagy-related 16-like and immunity-related GTPase M are part of the autophagy pathways that are important for cellular responses to IBD [28]. There is evidence that supports the hypothesis that inappropriate immune reactions to luminal bacteria are important in IBD pathogenesis, since all three genes seem to be involved in recognition and response to intracellular pathogens [28]. In addition, patients with Crohn's disease produce antibodies against the bacterial protein flagellin in those who have NOD2-associated disease, while anti-flagellin antibodies are uncommon in ulcerative colitis patients. Interestingly, the yeast *Saccharomyces cerevisiae,* has been shown to be a common inhabitant of the oral fungal community [29], and it may have a possible connection with IBD. Its relationship to IBD is largely unknown although patients with ulcerative colitis tend to lack antibodies to *S. cerevisiae*, which are usually present in Crohn's disease patients [28]. Another intriguing fact is that smoking (nicotine) seems to be protective for ulcerative colitis

but not for Crohn's disease [28], although the exact mechanisms operative here have not been fully elucidated, nor do we know the possible role that ingested food products play under these circumstances. In this regard, within the gut-microbiome-immune network, it has become clear [30] that, after passing through the colonic epithelium and mucosa, microbial metabolites, arising during the processing of ingested food products, can enter and accumulate in the vasculature, where they can elicit a wide range of biologic functions including those rendered by the immune system. Accordingly, such processes and responses can be seriously affected following the exposure to nicotine.

2.3. Bacterial and fungal pathogens affected by nicotine

In light of the foregoing, our laboratory began a series of experiments [31] that initially reexamined the effect that nicotine – perhaps the most important and potent, pharmacologically active substance in tobacco – has on microbial viability. We included some microorganisms known to naturally colonize the oral cavity and g.i. tract, as part of the human microbiome, which could impact on the host response to an infection occurring in such locations and/or provide antigenic stimulation/immunization. Proposed studies, such as these, involving direct exposure of bacteria to nicotine and determining what effect these interactions may have on immunity to infection and the inflammatory response (such as might occur in the bowel wall and periodontal disease) have rarely been done or reported in peer-reviewed publications. Yet they should provide new, important and key insights on nicotine-microbial-host immune system interactions.

Some of the key details on the procedures that we used along with the resulting findings have been published elsewhere [31] and are summarized as follows. Diverse groups of bacteria and fungi were mixed separately in vitro with various concentrations of commercially available, purified nicotine. These mixtures were then cultured onto agar plates for 24 – 48 h, and inhibitory activity was evaluated based on measuring colony-forming units. This is a well-accepted and relatively routine method for calculating the number of surviving organisms present in the original test mixtures. As shown in Fig. 1, nicotine caused a dose-dependent growth inhibition of a broad spectrum of our test organisms, some of which are known pathogens. Equally affected were both gram-positive and gram-negative bacteria, and the fungal organism, *Candida albicans*. Although not shown here, but published elsewhere [31] nicotine exerted a significant inhibitory effect against another fungal pathogen, *Cryptococcus neoformans*. Collectively, these two opportunistic fungi are frequent indigenous colonizers of the human oral cavity [29] and it has been well established that they are capable of causing superficial and systemic infections primarily in immunocompromised patients, or in people who are being treated with certain antibiotics (especially for *C. albicans*). In contrast, *Staphylococcus aureus* and *Mycobacterium phlei* were only slightly inhibited following nicotine exposure. Also significant, along these lines, was our finding that nicotine had strong inhibitory activity against the viridans *Streptococci* (a member comprising a large portion of the oral resident microflora that have been well documented as a significant cause of bacteremia and infective sub-acute endocarditis). Such a result could minimize the harmful effects associated with this group of organisms by limiting or preventing their dissemination, under certain conditions, from the mouth to the bloodstream. Levels of inhibition $\geq$ 50 % occurred when most of the affected organisms were cultured with nicotine at concentrations ranging from 100 to 250 u g/ml. It is noteworthy that these levels of nicotine can be found in vivo [32], especially in the oral cavity of smokeless tobacco users, thereby making these findings physiologically relevant.

3. Conclusion

Based on the foregoing and the results from our limited series of experiments [31] and those of others [7,8], the ability of nicotine to limit or interfere with the growth of various human microflora could be considered a significant finding. Such results could have broad range implications and relevance, since a large segment of the human population uses nicotine-containing tobacco products or nicotine alone for therapeutic purposes (withdrawal relief), and very little is known on how such events impact on various metabolic processes, especially those involving the microbiome and the host's immune system. A large body of evidence has revealed that IBD is most likely a result of aberrations (dysregulation) of mucosal immune reactivity initiated by one or more yet-to-be determined stimulus and/or etiologic agent(s) possibly involving one or more organisms that colonize the g.i. tract. Nicotine exposure, either through the use of gums or lozenges, especially in the oral cavity, where it occurs most often and would interact most intensely and directly with the contents of the oral cavity, could seriously affect or shift the type of species and/or the amount of microflora colonizing the mouth. Similar effects could manifest themselves in the

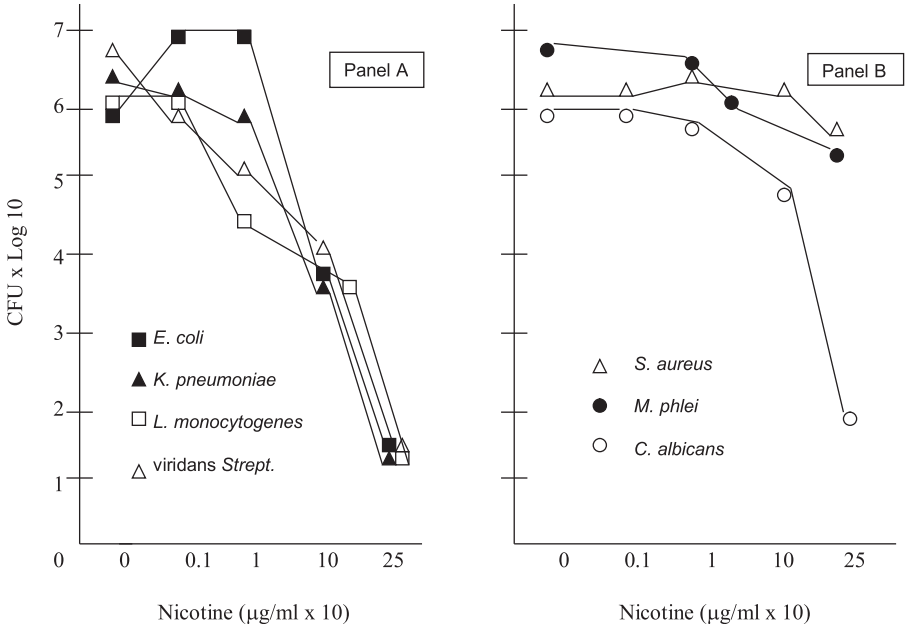

Fig. 1. (Panel A) Dose-dependent growth inhibition of the following bacteria: *Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes*, and viridans *Streptococci* by nicotine. Organisms were mixed with nicotine in vitro and cultured onto blood agar. After re-incubation, colony-forming units (CFU) of the number of surviving bacteria were counted. Each data point represents the mean value of 3 replicate experiments. (Panel B) Dose-dependent growth inhibition of the following bacteria and fungi: *Staphylococcus aureus, Mycobacterium phlei* and *Candida albicans*. Organisms were mixed with nicotine in vitro and cultured onto blood agar. After re-incubation, CFU of the number of surviving organisms were counted. Each data point represents the mean value of 3 replicate experiments.

g.i. tract and elsewhere following the use of the nicotine dermal patch, which leads to systemic absorption of nicotine. As a by-product of these events, degradation products of altered or dying organisms could contribute or modify the development of various pathologic processes such as periodontal disease(s) and IBD, as well as enable other microorganisms, including newly acquired pathogens, to proliferate and serve as foci for subsequent infections. On the other hand, nicotine exposure in the oral cavity could have a subtle beneficial effect on the host, by limiting the growth of certain respiratory / enteric or indigenous opportunistic pathogens that enter the body through the oral/nasal passages either as the result of inhalation of infectious aerosolized particles or via the ingestion of contaminated food products. As a follow-up to these provocative findings, future related studies should examine whether nicotine exerts its anti-microbial effects against a much broader range of indigenous microflora than has been studied so far, along with focusing on the molecular biologic mechanisms and host pathologic changes associated with nicotine-mediated killing of the oral and intestinal microflora.

Declaration of Competing Interest

The authors have none to declare.

Acknowledgements

This work was partially supported by funds provided by the Department of Biomedical Sciences, NYIT College of Osteopathic Medicine. The authors thank the publisher of the Journal of Medical Microbiology (JMM) for granting us permission to reuse in this paper, without being subject to any copyright infringement, some of the material previously published by one of us (CSP) in the JMM. We also thank Jane Pavia for contributing to the design of the graphical abstract.

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