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CORRESPONDENCE

Antimicrobial activity of nicotine against a spectrum of bacterial and fungal pathogens

Cigarette smoking and the use of smokeless tobacco continue to be relatively common practices in our society although such habits have been linked to serious illness and disruption of normal physiological processes [1]. Other related health risks may include a predilection to certain types of infections due to immune system dysfunction or selective killing of micro-organisms following exposure to tobacco products or nicotine, or both. The naturally occurring alkaloid, nicotine, is considered to be the addictive and most pharmacologically active substance among the many compounds present in tobacco products. The initial interaction of nicotine with the human body occurs most often in the oral cavity, where it would be expected to be most active and exposure to it most intense. Its possible effects on immunity (especially near mucosal surfaces) have been described only recently [2], while the effects of its interaction with the normal, indigenous microflora (especially in the oropharynx) is unknown; yet relatively high salivary concentrations (70–1560 μg/ml) of nicotine are achievable here for those using tobacco-based products [3]. Along these lines, little has been reported [4–6] on the ability of nicotine to support or suppress the growth of micro-organisms, and such studies have provided only inconsistent results. With these considerations in mind, a broth-dilution method was used in the present study to examine the effect that nicotine may have on the growth of various bacteria and fungi.

A pure nicotine solution (Sigma) 1 g/ml was diluted in phosphate-buffered saline (PBS) and tested at concentrations of 1.0, 10, 100 and 250 μg/ml. Each of the micro-organisms tested, except for *Borrelia burgdorferi*, were derived from stock cultures grown in trypticase soy broth (TSB) that were prepared from Cultiloops (Chrisope Technologies, Lake Charles, LA, USA). The B31 strain of *B. burgdorferi* was grown in BSK medium as described previously [7]. These late log-phase cultured bacteria and fungi were diluted separately to final concentrations equal to 1×10^7 bacteria/ml in BSK medium (for *B. burgdorferi* only) or 1×10^7 cfu/ml in TSB (for all others). Then, 50 μl of diluted organisms were mixed with 50 μl of the various concentrations of nicotine solution or of PBS control. These mixtures were added to separate, snap-cap tubes and co-incubated at 35°C for 4–8 h. At the end of the incubation period, all the mixtures except

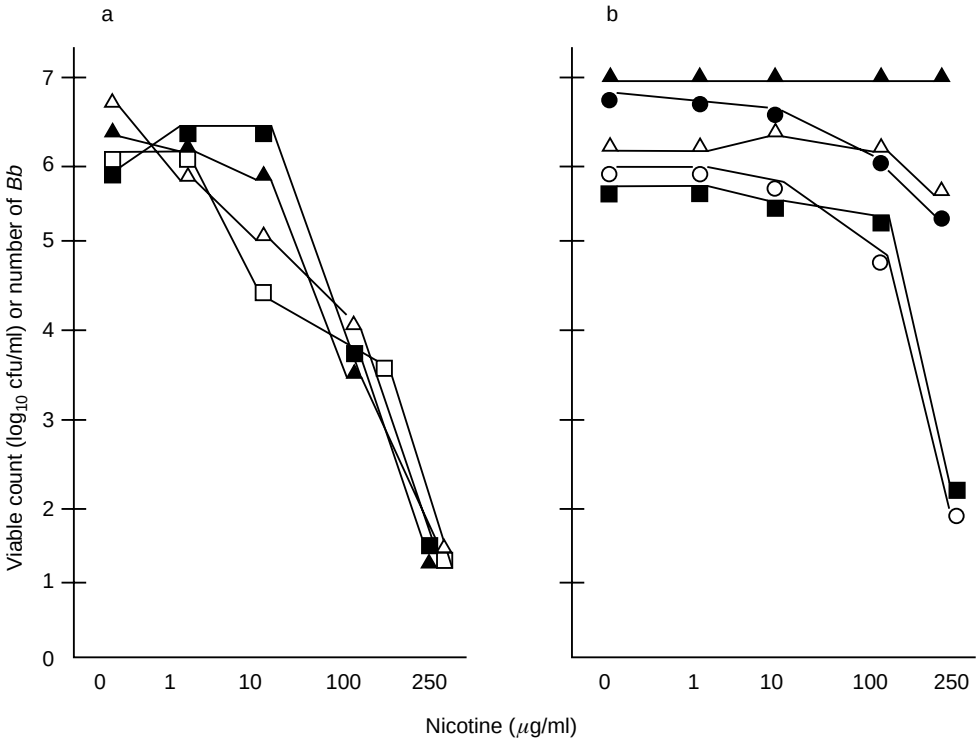

Fig. 1. Nicotine-mediated growth inhibition of various bacteria and fungi. Each point represents the mean value of three replicate experiments. (**a**) ■, *Escherichia coli*; ▲, *Klebsiella pneumoniae*; □, *Listeria monocytogenes*; △, Viridans streptococci. (**b**) ■, *Cryptococcus neoformans*; ▲, *Borrelia burgdorferi*; △, *Sraphylococcus aureus*; ●, *Mycobacterium phlei*; ○, *Candida albicans*.

the *B. burgdorferi*-nicotine mixtures were diluted 10-fold (from 1 in 10 to 1 in 1000) in PBS and 50-μl volumes were dispensed and spread on blood agar plates. After incubation for 24–48 h, the number of cfu was counted. Only plates with between 25 and 250 cfu were considered suitable for counting and these were used for calculating the number of surviving organisms present in the original test mixtures. The 100-μl *B. burgdorferi*-nicotine mixtures were supplemented with additional BSK medium (200 μl) (following the initial incubation period of 4–8 h), and were then re-incubated at 35°C. The number of motile (surviving) *B. burgdorferi* spirochaetes was counted microscopically 2–3 days later [7].

Nicotine caused a dose-dependent growth inhibition (Fig. 1) of a broad spectrum of the test organisms, some of which are known pathogens. Gram-positive and gram-negative bacteria were affected equally, along with the acid-fast *Mycobacterium phlei* and the opportunist fungi *Candida albicans* and *Cryptococcus neoformans*, thereby suggesting a common mechanism of action. Levels of inhibition $\geqslant$50% occurred when most of the affected organisms were cultured with nicotine at 100–250 μg/ml. It is noteworthy that such concentrations of nicotine can be found *in vivo* [3], especially in the oral cavity of smokeless tobacco users, making these findings physiologically relevant. It should also be noted that the viridans streptococci used in these experiments – which were also highly susceptible to the effects of nicotine – are an almost universal inhabitant of the oropharynx. In contrast, *Staphylococcus aureus* and the spirochaete *B. burgdorferi* (the agent of Lyme disease), were only slightly inhibited or were completely unaffected following exposure to nicotine.

The ability of nicotine to limit or interfere with the growth of selected micro-organisms was a significant finding in these experiments. Such results have a broad range of implications and relevance, as a large segment of the human population uses nicotine-containing tobacco products or nicotine alone for therapeutic purposes (withdrawal relief). Perhaps the greatest impact would be for those who use chewing-gum containing nicotine. Nicotine exposure, especially in the oral cavity, could seriously affect or shift the type of species or amount of microflora colonising the mouth, or both, as well as intensifying microbial penetration through an injured oral mucosa. If so, degradation products of dying organisms, after interacting with nicotine, could contribute or modify the development of periodontal disease(s) [8], as well as other inflammatory processes that might occur at mucosal surfaces, especially in the lower gastrointestinal tract such as inflammatory bowel disease [9]. Furthermore, such nicotine–microbial interactions could enable other microbes, including pathogens, to proliferate and serve as foci for subsequent infections. On the other hand, nicotine exposure could have a subtle beneficial effect on the host by limiting the growth of certain respiratory tract and enteric pathogens, as they enter the body through the oral and nasal passages.

Charles S. Pavia, Alexandra Pierre, John Nowakowski*, *Department of Microbiology, New York College of Osteopathic Medicine, New York Institute of Technology, Old Westbury, New York 11568 and *Division of Infectious Diseases, New York Medical College, Valhalla, NY 10595, USA (e-mail: cpavia@iris.nyit.edu)*

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