Cigarette smoking and the use of smokeless chewing tobacco have been closely tied to the development of several human cancers, including lung, oral, esophageal, and bladder. Additionally, individuals exposed to tobacco or tobacco smoke have a substantially increased risk of cardiovascular diseases, destructive periodontitis, and pulmonary, immunological, and gastric dysfunction. Although smoking has been a major public health issue for many decades, a fairly new concern is the use of smokeless chewing tobacco, which is becoming increasingly prevalent among U.S. teenagers and young adult males. Among the most well-characterized chemicals found in tobacco and tobacco smoke are polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene, and the highly addictive alkaloid, nicotine and its metabolites. Traditionally, views of tobacco-related carcinogenesis have been centered on genotoxic mechanisms, i.e., DNA damage/mutation inflicted by the binding of bioactivated and nucleophilic components such as the PAHs. However, with the realization that cancer is a multistep process, requiring many types of chemically mediated insults, came closer scrutiny of the additional, less obviously genotoxic, chemicals present in tobacco.

Nicotine, as one of the most biologically active chemicals in tobacco smoke and smokeless tobacco, has been the subject of intense scientific scrutiny. It is now known that nicotine, working through the neuronal nicotinic acetylcholine receptors (nAChRs) in the brain, is responsible for the addictive nature of tobacco use; the alkaloid has also been implicated in pathologies such as delayed wound healing and reproductive disorders. Studies on the cellular effects of nicotine have used a variety of functional endpoints in many different cell types. From this work, it has become apparent that nicotine binds to multiple receptors and activates several highly central signal transduction pathways. As a result, nicotine exerts diverse cell-type specific effects. To further complicate the picture, nicotine is converted, during the production of cigarettes and chewing tobacco, to two highly mutagenic nitrosamines, N’-Nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane (NNK), and is metabolized in vivo to cotinine. These chemical derivatives also exhibit a wide spectrum of biological activity as compared to the parent compound.

The cells of the oral cavity in smokers are intermittently exposed to very high concentrations of the component chemicals of tobacco and tobacco smoke. Due to the relevance of the oral mucosa/submucosa as one of the earliest targets and its importance in destructive periodontal conditions, previous investigators have explored the impacts of nicotine on human gingival fibroblasts (HGF) in culture. Nicotine-induced alterations have been demonstrated in such functions as production of cell surface and extracellular matrix proteins, proliferation, attachment to various surfaces, and chemotaxis; findings have been inconsistent as to whether or not these effects are more likely mediated through classical nAChRs or an alternative (unconventional) receptor. The strength of this month’s highlighted work by Argentin and Cicchetti lies in the fact that the investigators extend these previous studies and begin to address, in a carefully orchestrated manner, multiple important aspects of nicotine toxicity/carcinogenicity in this highly relevant cell type. This work, which is carried out at physiologically relevant concentrations in vitro, examines the impact of the alkaloid on cell proliferation, apoptotic cell death, DNA integrity, and cellular redox status. The careful execution of these studies allows ties to be made among the diverse actions of nicotine and begins to address important mechanistic issues in its toxicological effects.

Although a great deal of work has been carried out on the mutagenicity of the common PAHs found in cigarettes, only a few studies have addressed the genotoxic nature of nicotine itself. Although tests examining point mutagenicity have generally been negative, the alkaloid has been shown in some cases to increase chromosome aberrations in exposed cells (Trivedi et al., 1990). However, findings in this regard have been somewhat inconsistent, thus highlighting the need for

1 For correspondence via fax: (970) 491-8304, E-mail: julie.campain@colostate.edu.

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further exploration. In attempts to clarify this issue, Argentin and Cicchetti have examined the ability of nicotine to induce micronucleus formation (as a measure of chromosomal DNA damage) in HGF. Dose-response, as well as time-course studies, substantially add to the utility of this data set. The induction of DNA damage observed by the authors in HGF was rapid, occurring at maximum levels by 24 h exposure to nicotine, and prolonged, lasting throughout 72 h of treatment. In addition, the maximum level of damage was detected at 1 μM, a concentration of nicotine that demonstrated minimal effects on HGF viability and that has previously been reported in the plasma of tobacco users (Russell et al., 1980). Interestingly, higher concentrations of the alkaloid were less effective at inducing chromosomal abnormalities, in part due to the induction of non-apoptotic cell death in treated populations.

Nicotine has also been implicated in free radical generation in rodent and human cells of various types. In further mechanistic studies described in the highlighted article, Argentin and Cicchetti were able to show, for the first time, that nicotine induces a significant amount of reactive oxygen species (ROS), as measured by the fluorescent probe, 2',7'-dichlorofluorescein, in exposed HGF. One of the more noteworthy series of experiments described in this article directly addresses the relationship between ROS induction and the observed DNA damage. In cells pretreated with two antioxidants, N-acetylcyesteine and catalase, nicotine was substantially less effective at inducing either oxidative damage or formation of micronuclei, thus demonstrating linkage between the two pathological effects. The fact that N-acetylcyesteine was more effective at protecting against nicotine-induced damage suggests that replenishment of glutathione stores, as well as scavenging of oxygen radicals, was important.

Depending on the cell type, nicotine has been shown to induce or inhibit the process of programmed cell death. In support of the latter, Argentin and Cicchetti also demonstrate in their article that nicotine acts as an inhibitor of staurosporine-stimulated apoptosis in HGF. Given the multistep nature of malignant transformation, it is possible that abrogation of the apoptotic pathway in nicotine exposed cells could be important in terms of smoking (and chewing tobacco)-associated cancer of the lung and oral cavity. Due to the presence of potent mutagens in tobacco and tobacco smoke, tissues chronically exposed would harbor a substantial population of genetically abnormal or “initiated” cells. Under normal conditions, the majority of these mutant cells would undergo apoptosis, thus preventing their further malignant progression. Nicotine, through inhibition of programmed cell death, could serve to effectively promote the clonal expansion of chemically initiated cells. Further, the clastogenic activity of the alkaloid, as measured by these investigators, could potentially aid in development of genetic instability and thus, favor conversion to the fully malignant phenotype. In essence, the complex chemical mixture found in cigarettes could act as a highly effective complete carcinogen. One way to address this issue in the future is through quantitative studies characterizing the carcinogenic potential of PAHs in the presence and absence of promoting agents such as nicotine. Further, abrogation of the apoptotic pathway by nicotine could subsequently decrease the efficacy of anti-cancer regimens (many of which rely on induction of apoptosis in damaged cells) in individuals who continued to smoke during therapy. Support for this supposition comes from the highlighted work by Argentin and Cicchetti in that, even in HGF with extensive nicotine-mediated DNA damage, the proliferation rate did not change and the normal pathway responding to cell injury, apoptosis, was not activated.

Although this article does not explore gene expression changes or alterations in signal transduction pathways that might be involved in the observed effects of nicotine on HGF, several previous studies in other cell types provide mechanistic clues and suggest future areas of investigation. Arredondo et al. (2003), studying potential causes of nicotine-induced dermatologic dysfunction and premature aging, found that acute exposure to the alkaloid increased expression of several cell cycle regulatory proteins, including p21, the anti-death protein, B cell lymphoma gene-2 (bcl-2), and caspase 3, in closely related human dermal fibroblasts. Additional alterations were seen in the expression of dermal structural components and proteolytic enzymes of the extracellular matrix. Additionally, chronic exposure to nicotine also altered the ligand-binding kinetics and subunit composition of the nAChR in these cells, thus modulating the effects of the important endogenous transmitter, acetylcholine, on normal cutaneous function. Several investigators have demonstrated that nicotine antagonizes the induction of apoptosis by opioids in lung cancer cells. Pro-growth pathways activated by nicotine in these cells include the mitogen-activated protein kinase (MAPK), the protein kinase C, and the Akt pathways, all of which are thought to lead to overexpression or increased activity through phosphorylation of bcl-2 (Heusch and Maneckjee, 1998; Mai et al., 2003; West et al., 2003). In contrast, in work described by Onoda et al. (2001), nicotine-mediated reduction in the apoptotic response of head and neck cancers to cisplatin and UV light was linked to the p38 and c-Jun NH2-terminal kinase-1 (JNK1) pathways, as well as the growth arrest pathway associated with gadd153.

Although many investigators have identified individual gene expression changes in nicotine-treated cells, the complex actions of the alkaloid suggest that technologies for global expression analysis will yield a more complete and accurate picture of molecular mediators in different cell types. Several investigators have begun to take this approach, using microarray technology for analysis of gene expression patterns in nicotine-exposed versus control cells or tissues. Li et al. (2002), in a particularly interesting study, utilized microarray analysis to perform mRNA expression profiling in specific regions of the rat brain associated with chronic nicotine exposure and addiction. Families of altered genes highlighted as to
potential roles in inhibition of apoptosis and neuroprotection were (1) those involved in the phosphatidylinositol signaling pathway and calcium homeostasis, (2) growth factor receptors and cytokines, (3) the NF-κB and JNK signaling pathways, and (4) genes involved in remyelination and axonal growth.

The findings from these varied studies are interesting and indicate that nicotine is capable of working through several different signal transduction pathways to exert its diverse effects on target cells, especially with regard to apoptosis. Which pathway is affected appears to depend on the administered dose, the agent used to induce apoptosis (and its mechanism of toxicity), or the cell type being examined; it is presently unclear what variable, if any, is most important. Certainly, the type of nicotine receptor(s) present on target cells will have a major impact on tissue-specific pathological effects of the alkaloid. As two of the primary activities of nicotine in most cell types are modulation of the phosphatidylinositol pathway and increasing intracellular calcium levels, it is a highly bioactive substance, activating many kinase-associated signal transduction pathways. In the highlighted article by Argentin and Cicchetti, reversal by nicotine of the action of staurosporine, which acts to induce apoptosis through fairly nonspecific inhibition of protein kinases, is not unexpected and suggests future, more detailed, avenues for study. More specific protein kinase inhibitors, especially those that target distinct protein kinase C isoforms, may shed some light on whether this pathway is, as suggested by the authors, central to the activities of nicotine. Furthermore, detailed analysis of the signal transduction pathways altered in a single cell type, i.e., HGF, by diverse agents that induce apoptosis and the antagonistic actions of nicotine to reverse these changes may provide clear linkage between specific types of cellular dysfunction and the respective molecular mediators.

There is one important caveat to the highlighted studies carried out by Argentin and Cicchetti. In humans, cancers of the oral cavity arise from cells of epithelial, and not mesenchymal, origin. Thus, the HGF studied by these investigators would not themselves be the “initiated” precursor leading to ultimate formation of a malignant tumor. However, as discussed by Giannopoulou et al. (2001), epithelial cells and fibroblasts of the oral mucosa are in very close association with one another and undoubtedly influence each other’s viability and function. Under normal physiological conditions, the epithelial cells act as a barrier to noxious substances and pathological microorganisms in the mouth. The fibroblasts, in turn, help produce the extracellular matrix, which plays a growth regulatory role for the overlying epithelial cells. These investigators have demonstrated that interaction of nicotine-treated epithelial cells with untreated gingival fibroblasts is detrimental to the latter, in that both cell proliferation and production of collagen and noncollagen proteins were decreased in a dose-dependent manner. One hypothesis discussed by these investigators was that nicotine impacted fibroblast function indirectly by altering the metabolism of the treated epithelial cells, potentially changing the repertoire of diffusible growth factors and cytokines produced and excreted. With regard to development of cancer, Hanahan and Weinberg (2000) discuss “heterotypic” signaling between the diverse cell types in a tumor, emphasizing the importance of abnormal activity on the part of fibroblasts, endothelial, and immune cells in promoting survival and growth of the tumor cell, itself. Future studies by Argentin and Cicchetti and other investigators could potentially address whether these same pathological effects of nicotine measured in HGF, i.e., oxidative stress, DNA damage, and inhibition of apoptosis, are seen on the epithelial cancer “target” cell. Additionally, molecular alterations in the gingival fibroblast treated with nicotine, and their potential role in promoting transformation of associated epithelial cells may also provide fruitful avenues for exploration.

REFERENCES


