

MINI-REVIEW ARTICLE

NITRIC OXIDE-MEDIATED PATHOGENESIS DURING NICOTINE AND ALCOHOL CONSUMPTION

R. G. COOPER^{1*} AND T. MAGWERE²

*¹Division of Physiology, Faculty of Health,
Birmingham City University, 701 Baker Building,
Franchise Street, Perry Barr, Birmingham B42 2SU, U.K.*

and

*²St. James University Hospital,
Cancer Research Building,
Beckett Street, Leeds LS9 7TF, U.K.*

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Abstract : Nitric oxide (NO) is formed by different cell types in response to a variety of physiological and patho-physiological stimuli. The intake of nicotine and/or alcohol has patho-physiological effects on organ function, and the progression of alcohol-/tobacco-related diseases seem to be directly influenced by NO-mediated mechanisms. Nicotine has an adverse influence on blood vessel functionality, repair and maintenance. Chronic nicotine exposure augments atherosclerosis by enhancing the production of pro-inflammatory cytokines by macrophages which then activate atherogenic NF-kB target genes in aortic lesions. Alcohol produces NO which speeds up the apoptosis of neutrophils. Alcohol sensitizes the liver to endotoxemic shock. Nitrosative stress and increased basal levels of NO contribute to tumour growth. The progression of disease seems to be directed via a definite NO-mediated mechanism. This review gives an insight into how intake of tobacco and alcohol may affect quality of life.

Key words : alcohol cancer disease
ethanol nicotine nitric oxide
nitrosative stress oxidative stress pathogenesis
reactive oxygen species tobacco

INTRODUCTION

Nitric oxide (NO) is formed by different cell types in response to a variety of physiological and pathophysiological stimuli. Its biological role was first realised in 1987

when Palmer and co-workers discovered that the physiological effects of an 'endothelium-derived relaxing factor' (EDRF) were due to NO (1). NO is enzymically generated from I-arginine by a unique family of calcium/calmodulin-binding NO synthases (NOS) now

*Corresponding Author: Dr. R.G. Cooper, Senior Lecturer in Physiology, D.Phil., Phone : +44 121 331 6227; Fax : +44 121 331 5498; E-mail address : rgcooperuk@yahoo.com

identified as neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) isoforms (2). nNOS and eNOS are constitutively expressed in tissues whereas iNOS is present in all nucleated mammalian cells and is inducible by endotoxins or cytokines. NO has an important signalling role in mammalian cells but also mediates tissue injury in pathophysiological states as diverse as septic shock, hypertension, stroke, and neurodegenerative diseases (3, 4, 5). The toxicity/tissue damage caused by NO *in vivo* is attributed to the production of peroxynitrite (ONOO⁻) and other reactive nitrogen species (RNS) produced when NO reacts with superoxide ions during conditions of oxidative stress (6).

Lifestyle and social pressure often result in the concurrent intake of alcohol and nicotine. To date, a laboratory study of ethanol and nicotine on renal function, and a review of the effect of tobacco smoking on renal function have been published (7, 8). Searches on PubMed and Medline for articles published over the last two decades with impact factors greater than 0.5 were selected for inclusion in the article. In this mini-review we discuss the influence of nicotine and ethanol on NO-mediated pathogenesis. We also provide further evidence compiled from the literature implicating NO as the "messenger of death" in tobacco- and alcohol-related diseases.

Impairment of genes coding for NOS

The endothelial NO synthase (eNOS) gene may play a part in the development of blood pressure and left ventricular mass from childhood to early adulthood (9). It has been demonstrated that variants in the eNOS gene are associated with an increased risk of

plaque on carotid arteries (10). Nicotine alters the expression of endothelial genes including eNOS; this has an adverse effect on vascular tone regulation and thrombogenicity (11). Genetic-mediated mechanisms of abnormal nerve impulse generation arise from NO alterations in neuronal function within the brain. NO disrupts the gene for iNOS thus reduces progression of cerebral aneurysms (12). Attenuated NO production and an increase in the production of endothelium-derived contracting factor (EDCF) are the principal causes of impairment in large arteries, while a decrease in endothelium-dependent hyperpolarisation and increased release of EDCF are the main causes of small artery impairment (13). Endothelin peptides are strong vasoconstrictors involved in the pathogenesis of cardiovascular disease. Endothelin2-expression causes normotension as a consequence of NO formation (14). We suggest therefore that NO-mediated disruptions in central nervous system vasculature inducible by nicotine and/or alcohol intake may have serious chronic consequences on health. Clearly nicotine has an adverse influence on blood vessel functionality, repair and maintenance.

Influence of nicotine

Nicotine stimulates release of nor-adrenaline in the paraventricular nucleus and amygdala via direct influence on the nucleus tractus solitarius glutamate afferents and N-methyl-D-aspartate receptors that partly stimulate NO production with consequential activation of nor-adrenergic neurones (15). Glutamate release is significantly elevated following acute nicotine injection (16).

Chronic nicotine exposure augments atherosclerosis by enhancing the production of pro-inflammatory cytokines by macrophages which then activate atherogenic nuclear factor kappa-B (NF-kB) target genes in aortic lesions (17). Nicotine may also potentiate lipopolysaccharide (LPS)/interferon-gamma-induced cytotoxic effects by enhancing NO production and enhancing iNOS gene expression, indicating a strong association between inflammation and smoking (18).

Passive smoking produces selective impairment of neurogenic and endothelium-dependent relaxation of corpus cavernosum smooth muscle via attenuated NO synthesis or availability (19). Passive smoking also impairs endothelium-dependent relaxation of arteries mediated partly through the degradation of released NO via superoxide anions derived from cigarette smoke (20). Nicotine may potentiate noradrenaline-induced vasoconstriction (21) thus reducing blood flow to organs. NO antagonises the ulcerogenic action of nicotine via a cytoprotective way (22). Nicotine reduced angiogenesis in the gastric mucosa via inhibition of NO synthesis and halting cell renewal processes (23). Smoking also impairs the spontaneous and drug-induced healing of ulcers (23).

Nicotine and oxidative stress

Nicotine absorption in a group of bidi workers chronically exposed to tobacco dust was shown to be associated with increased serum levels of NO and markers of oxidative stress (24) suggesting a mediating role for NO in lung-related diseases. In rat mesencephalic cells in culture, nicotine was

again shown to dose-dependently induce intracellular ROS levels leading to activation of stress-dependent NF-kB pathway (25). Another recent study has also reported that acute infusion of nicotine impaired the nNOS-dependent dilatation of cerebral arterioles through induction of oxidative stress (26). Together these results suggest that exposure to nicotine has deleterious implications for the development of CNS diseases. Table I summarises the results of some studies showing the involvement of nicotine in oxidative stress.

Nicotine and cancer

Nicotine stimulates angiogenesis and promotes tumour growth and atherosclerosis in mouse models and both processes are thought to be mediated via endothelial production of NO, prostacyclin, and vascular endothelial growth factor (VEGF) (27). At physiologically relevant concentrations, nicotine was shown to cause DNA damage and prevented staurosporine-induced apoptosis in human gingival fibroblasts through induction of iNOS expression *in vitro* (28). Nicotine may also have a role in the development of oral cancer through inhibition of NO-induced apoptosis in epithelial cells (29). Nitric oxide radicals released from tobacco-related compounds were shown to cause nitrosative stress and DNA strand breaks in immortalized hamster cheek pouch cells (30). Taken together, results from these studies implicate nicotine and NO in the development of oral and lung cancers.

Influence of alcohol

Moderate consumption of red wine

TABLE I: Summary of studies showing an association between nicotine and oxidative stress.

<i>Experimental model of subject</i>	<i>Nicotine concentration</i>	<i>Main finding</i>	<i>Reference</i>
Rat oesophageal mucosa	50–800 ng/mg protein/ml	Increased lipid peroxidation and ROS in homogenates.	Wetscher et al. (57)
Rat pancreatic tissue	200–800 ng/mg protein/ml	Increased lipid peroxidation and free radicals in homogenates.	Wetscher et al. (58)
Rat	3.5 mg/kg body weight	Increased lipid peroxidation and decreased activities of superoxide dismutase, catalase, and glutathione reductase.	Ashakumary & Vijayammal (59)
Rat mesencephalic cells	0.1–10 μ M	Dose-dependent increase in ROS levels, activation of NF-kB.	Barr et al. (25)
Human (bidi workers)	unknown, chronic (10–24 y)	Increased lipid peroxidation and NO in serum, plus decrease vitamin C, RBC-SOD and total antioxidant capacity in blood.	Swami et al. (24)

improves ischemia-induced neovascularisation in high cholesterol conditions by increasing the number and functional activities of endothelial progenitor cells and by restoring the eNOS-NO pathway (31). Chronic ethanol ingestion, however, induces aortic endothelial oxidative stress and down regulation of NO generation leading to vasorelaxation and hypertension (32). Binge drinking in patients with cirrhosis show no increase in NO levels in serum (33) suggesting the liver has a crucial role in synthesis of NO during alcohol consumption, and acute food intake (34). Alcohol consumption with food slightly attenuates serum NO levels (34).

Immune function may be reduced via the stimulation of apoptosis of neutrophils accelerated by ethanol and mediated via the generation of NO (35). This may potentially predispose the chronic alcoholic to recurrent bacterial and viral infections.

Ethanol may augment oxidative damage in the adenohypophysis via over-production of NO and carbon monoxide (36). Ethanol-induced pathology is further expressed in the modulation of the cytokine-induced iNOS expression in human astroglia (37) and inhibition of the activator of transcription-1 (38). Ethanol aggravates the pathomorphological consequences of NO generation associated with circulatory failure as a consequence of haemorrhage or sepsis, and adversely affects blood-brain barrier permeability (39).

Alcohol and oxidative/nitrosative stress

The metabolism of alcohol is inherently associated with the production of both reactive oxygen and nitrogen species (ROS and RNS) resulting in oxidative and nitrosative stress (40). Nitric oxide plays a mediating role in alcohol-related diseases and tissue damage including cancer (41, 42)

and oxidative liver injury (43). The work of Polikandriotis and colleagues highlights mechanisms through which chronic alcohol consumption might be involved in incidences of acute respiratory distress syndrome and lung injury. They first showed that chronic ethanol stimulated NO production via PI-3 kinase and hsp90-dependent induction of eNOS in porcine pulmonary artery endothelial cells (44). Using rat models they further showed that alcohol increased hydrogen peroxide production, eNOS expression and activity, as well as markers of oxidative stress and damage in lungs of male Sprague-Dawley rats (45, 46). In Caco-2 intestinal cells *in vitro*, ethanol was shown to induce iNOS expression as well as NO synthesis resulting in oxidative/nitrosative stress and disruption of microtubules formation and intestinal barrier function (47). The relationship between chronic ethanol, oxidative stress, and tissue injury has thus been established. Some results have however shown negative effects of ethanol on eNOS expression; chronic alcohol downregulated eNOS protein levels and combined synergistically with LPS to lower eNOS activity (48) and transcription (49) in male Sprague-Dawley rats suggesting that alcohol consumption might sensitize the liver to endotoxemic shock.

Alcohol and cancer

The association between alcohol and various cancers has been known for close to a century (41) and a detailed discussion of this topic is beyond the focus of this mini-review. We however focus on providing some evidence for the involvement of NO in alcohol-related cancers. It has been demonstrated in rat models that chronic

ethanol consumption increases NO production in organs such as the liver through induction of iNOS (50); the resulting nitrosative stress and increased basal levels of NO are likely to contribute to the promotion of tumour growth. NO plays an important role in angiogenesis and vascular function (51, 52) which are two important processes during tumour development and progression. NO and NO-derived RNS induce oxidative and nitrosative stress in cells causing DNA strand breaks while inhibiting DNA repair enzymes (51). A recent study in humans has shown that NO levels are elevated in serum of head and neck squamous cell carcinoma patients compared to controls (53). We suggest that the stimulation of NO production by ethanol is therefore likely to play an important role in the etiology of some cancers that preferentially rely on NO signalling.

Concurrent intake of alcohol and nicotine

Concurrent intake of nicotine and ethanol produce a complex central association as the nicotine-induced changes in glutamate and arginine secretion in the nucleus accumbens exert a possible modulatory effect on the former's release during the initial stages of chronic ethanol withdrawal (16). Indeed, the interaction between ethanol and nicotine, may progress via modulation of cerebella NO and cGMP (54). There is a possible functional correlation between the former and ethanol-induced motor impairment suggesting NO as a factor in cross-tolerance between nicotine and ethanol (55).

Concurrent consumption of nicotine and ethanol significantly exacerbates the risk of developing gastric ulcers via reduction in

mucus secretion, increased leukotriene B4 levels, increased activities of iNOS, xanthine oxidase and myeloperoxidase, and the expression of adhesion molecules in the gastric mucosa (56). The intake of nicotine and alcohol (alone and in combination) is also likely to precipitate or exacerbate pathological conditions in which ROS and RNS have been implicated to play a damaging role.

Conclusion

This review gave an insight into how two commonly-consumed and socially-accepted substances, tobacco and alcohol, are likely to impact on quality of life. The intake of alcohol and nicotine (either alone or in

combination) clearly has patho-physiological effects on organ function, and the progression of alcohol-/tobacco-related diseases seem to be directly influenced by NO-mediated mechanisms. We advocate suggestions of changes in life-style habit especially when alcohol and tobacco are taken concurrently with medication, and in diseases associated with ascites/cirrhosis, cardiovascular aberrations, and progressive renal failure. The influence of nicotine and/or ethanol on neuronal/endocrine-mediated pathways cannot be ignored.

Conflict of interest

None recorded.

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