REVIEW ARTICLE

Molecular Pathways for Nasopharyngeal Carcinoma focused on Acetaldehyde, Nitrosamines and Nicotine Exposures

Rabiatul Basria S.M.N. Mydin[1], Simon Imakwu Okekpa[1, 2]

[1] Oncological and Radiological Sciences Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Bertam, Kepala Batas, Pulau Pinang, Malaysia
[2] Department of Medical Laboratory Science, Faculty of Health Sciences, Ebonyi State University, Abakaliki, 840001 Ebonyi state, Nigeria

ABSTRACT

Recently, one of the head and neck tumours located at the nasopharynx epithelium known as nasopharyngeal carcinoma (NPC) have been associated with few cancer-promoting compounds that derived from alcohol, salt preserved foods consumptions and tobacco smoking such as acetaldehyde, nitrosamine, nicotine. These cancer-promoting compounds present the ability to damage the genome and disrupt cellular metabolic processes. This review will discuss further on the molecular mechanism of acetaldehyde, nitrosamine, nicotine and NPC risk. Acetaldehyde can exert influence as carcinogen macromolecular adducts to cellular proteins and DNAs whilst nitrosamines that commonly found in preserved salted foods/diets can contribute as a powerful carcinogen via endogenous nitrosation and reactives molecules by CYP2E1. Nicotine present in tobacco could reacts with nitrosamine to form NNN and NNK known as carcinogenic agent. NNK mediates unstable reactive oxygen species that can induce DNA lesion (α -hydroxylation of NNN at positions 2′and 5′) and microenvironment alteration for tumorigenesis. In conclusion, this study suggests acetaldehydes, nitrosamine and nicotine may contribute to NPC tumourigenesis.

Keywords: Acetaldehyde, Nitrosamines, Nicotine, DNA adducts, Nasopharyngeal carcinoma

Corresponding Author:
Rabiatul Basria S.M.N. Mydin, PhD
Email: rabiatulbasria@usm.my
Tel: +604-5622351

INTRODUCTION

Nasopharyngeal cancers (NPC) malignancy is rare in most part of the world but highly predisposed in Asian endemic (1). NPC is found in the epithelium of the nasopharynx and the most common type of oral cancers in certain populations (2-5). Numerous studies have debate on the NPC risk association with alcohol (acetaldehyde), smoking (nicotine) and salt preserved foods/diets (nitrosamines) consumptions (6-15). Alcohol (Ethanol) is metabolized into acetaldehyde (AA) through oxidative processes catalyzed by alcohol dehydrogenase (ADH), cytochrome P450 2E1 (CYP2E1) and other catalase. AA adheres to proteins and DNAs to generate DNA carcinogenic adducts causing DNA repair inhibition, DNA methylation and ROS formation (16). Assimilation of AA induces nasopharyngeal carcinoma because of the binding of AA to DNA and cellular proteins triggering the cellular function impairment leading to a cascade of immunological reactions (17). Most cancers delineated on the acetaldehyde exertion mainly occur at the oral cavity, pharynx and larynx (17). A part than that, it has been reported that intake salt preserved foods/diets such as salted fish, salted meat and preserved vegetables contains nitrosamines and nitrite that could lead to NPC risk (18). Nitrite is naturally not carcinogenic but becomes a carcinogen through endogenous nitrosation where the nitrite reacts with amides and secondary amines to produce nitrosamides and nitrosamines which present as oncogenic agent (19, 20). The potent of nitrosamines for carcinogenicity correlates with frequent or continuous dietary pattern (20, 21).

Cigarette smoking present as a very high risk factor for sino-nasal cancers, nasopharyngeal cancers, and oral cavity cancers (22). Tobacco smoke contains toxic compound such as nitrosamines (NNK & NNN), polycyclic aromatic hydrocarbons (PAHs), aromatic amines, volatile hydrocarbons, aldehydes, nitro compounds, phenols and inorganic composites (23, 24). Furthermore, nitrosamines association with nicotine is devastating to human. During smoking process, nicotine is transformed into NNAL, NNN and NNK which are highly toxic (25). Nitrosamines contained in tobacco substances are generated through a process known as nicotine and tobacco alkaloid nitrosation. Nitrosation of nicotine yields NNK and NNA. NNK found in tobacco smoke is pro-carcinogenic but requires CYPs activation in order to yield metabolites that are DNA reactive prompting pyridyloxobutylation,

pyridylhydroxybutylation and methylation of DNA nucleobases to generate DNA adducts (26–30). Note that the carcinogenic impact of acetaldehyde, nitrosamines and nicotine could also present in the certain population diets or lifestyle.

ACETALDEHYDE (AA): DNA, PROTEIN AND LIPID ADDUCTS

CYP2E1 metabolized acetaldehyde via NADPH-dependent, an acetaldehyde oxidizing system (31). Dysregulation of these acetaldehyde oxidative mechanisms could results in the accumulation of acetaldehyde which are toxic to human cells as described by Fig 1. Acetaldehyde is electrophilic and its electrophilic nature enables it to form adducts through chemical covalent bonding of products to DNA, proteins and lipids (32-38). Mutagenic effects of acetaldehyde is exerted through direct interaction with DNA, leading to lesion such as mutation and massive chromosomal damage. Induction of mutation in the hypoxanthine-phosphor-ribosyl-transferase gene (HPRT1) by acetaldehyde causes impairment in DNA synthesis and repair through deletion of nucleotide and elimination of DNA repair processes. Acetaldehyde could also induce DNA damage by exerting exchange of sister chromatids (18, 39, 40). Another DNA adduct induced by acetaldehyde is N2-propano-2′-deoxy-guanosine which is genotoxic as well as mutagenic and has the capacity of generating secondary lesions and consequent cross links between inter strands thereby impairing DNA replication to promote cell death (32). It also triggers replication errors, oncogenic mutations and onco-suppressor genes mutation thereby promoting carcinogenesis (38). Furthermore, N2-ethyl-deoxy-guanosine (N2-Et-dG) has been previously detected in acetaldehyde/alcohol mediated cancers of the head and neck (45-48).

Protein adducts formation by acetaldehyde occurs through the interaction with epsilon amino group in the lysines and alpha-amino group of the N-terminal amino-acids (41). This acetaldehyde adducts causes alteration in the function and structure of proteins. For instance, formation of acetaldehyde adducts with methyl-guanine

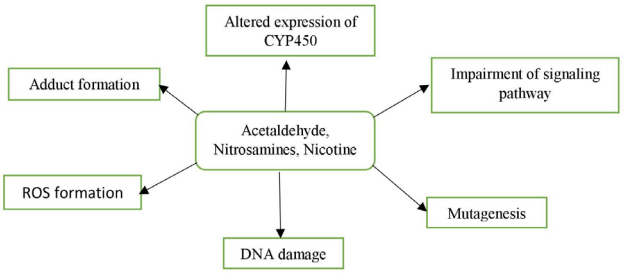

Figure 1: Potential molecular mechanisms of cancer-promoting compounds such as acetaldehyde, nitrosamine, nicotine. Example: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N′-nitrosonornicotine (NNN). 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL).

catalyzed by methyltransferase enzyme results in impairment of repair mechanisms of DNA which could induce carcinogenesis (38). Protein adducts causes impairment of catalytic reactions and consequent impairment of the function of NADPH-dependent CYP2E1 leading to more accumulation of acetaldehyde as described by Fig. 2.

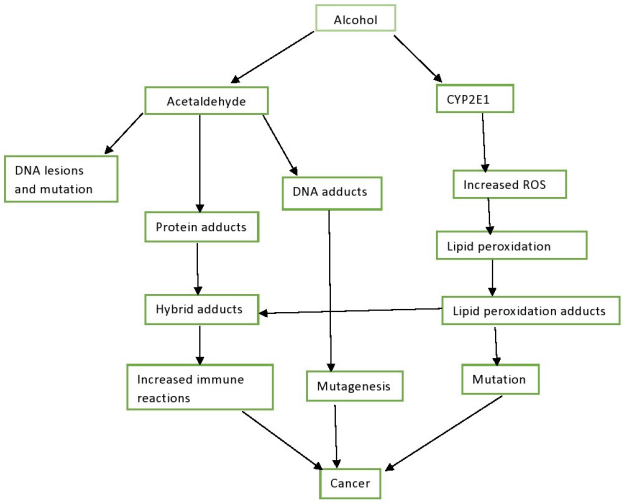

Figure 2: Schematic described the possible molecular mechanisms of Acetaldehyde-Mediated Nasopharyngeal Carcinoma (NPC). Alcohol is metabolized into acetaldehyde (AA) which stimulate pathways for carcinogenesis. AA then cause direct lesions on the DNA with resultant mutations. AA forms indirect covalent bonds with proteins to impair DNA repair mechanisms, and increase carcinogenesis potential. AA reacts with DNA to form DNA adducts. Persistent consumption of alcohol induces CYP2E1 resulting in reactive oxygen species (ROS) formation and consequent lipid peroxidation proceeded by DNA adducts formation. AA-protein adducts rejoin with DNA adducts to form hybrid adducts.

Acetaldehyde binds to glutathione triggering oxidative stress which progressed to lipid peroxidation (42). Lipid peroxidation mediates acetaldehyde induced carcinogenesis (43, 44). Induction of CYP2E1 in chronic alcoholic drinkers potentiate reactive oxygen species (ROS) generation resulting in the perpetuation of oxidative stress plus consequent cell death (32, 38, 40, 49). The generated ROS radicals (hydroxyethyl radical & superoxide anion) react with DNA, lipids and proteins to form adducts. ROS stimulates lipid peroxidation products such as 4-hydroxynonenal (4-HNE) and malondi-aldehyde (MDA) to be formed (Fig. 2) (38, 40). Aldehydes generated in cells cross-react to generate hybrid adducts. Combinations of MDA-acetaldehyde with protein adducts forms malondialdehyde-acetaldehyde (MAA) hybrid adducts (Fig. 2). This hybrid adducts acts synergistically to increase carcinogenic activities of various adducts (34, 49). Hybrid adducts perpetuate genotoxicity by stabilizing protein adducts (50).

Presence of acetaldehyde reduces the ability of the hepatocytes to metabolize carcinogens such as nitrosamines leading to accumulation of nitrosamines

in the peripheral tissue activated by CYP2E1 in the mucosa. Inducible CYP2E1 expression exposes the peripheral tissues to pathogens and carcinogenic substances due to low specificity of the nitrosamines to CYP2E1 (51) and generates harmful acetaldehyde which is highly reactive in tissues which results in oxidative stress influences, thereby exposing the peripheral epithelium to carcinogenic metabolites such as aflatoxin, aromatic hydrocarbons and nitrosamines (51). Therefore, acetaldehyde is considered as potential carcinogen which also commonly associated with upper aerodigestive cancers.

NITROSAMINE FROM SALT-PRESERVED FOODS

Furthermore, few vivo studies have revealed that salted fish consumption associated with the source of nitrosamines and plays an etiological role in the oncogenesis of NPC (52, 53). Diethyl-nitrosamine and dimethylnitrosamine (DMNA) have previously been tested in more than 20 species of human and non-human primates and it was discovered kindred with tumour induction. For nitrosamine fed rats, the tumour induction seen started at the nasal cavity. Nitrosamines is metabolized into reactive carcinogenic molecules by CYP2E1 and are expressed mainly in the epithelium of the nasal cavity of animals including humans. Exposure of humans to already formed N- nitroso compounds (NOC), nitrosating agents and precursors react in vivo to generate diazo compounds and carcinogenic NOC. Some bacteria reduces nitrate to form nitrite, which is further converted into carcinogenic N-nitroso compounds (54).

In addition, nitrite and other nitrosating agents can endogenously be synthesized by bacteria mediated enzyme reactions, activated neutrophil and macrophages (54). Neutrophil and macrophages generate nitric oxide radical through a reaction catalyzed by nitric oxide synthase. This generated nitric oxide radicals is cytotoxic and believed to contribute in the generation of carcinogenic nitrosamines, deamination of DNA base and oxidative damage (54).

The processes employed in preserving fish and other diets with salt are very inefficient and causes partial putrefaction of the diets and fish, thereby, triggering high levels of nitrosamines accumulation which are carcinogens in human and other animals (55-59). Salt preserved fish may possess Epstein-Barr virus (EBV) reactivating substances, bacterial mutagens and direct geno-toxins which all contribute to the association with NPC risk (60-61).

NICOTINE AND NITROSAMINES IN NPC DEVELOPMENT

Tobacco specific nitrosamines, induces formation of DNA adducts, protein adducts and lipid adducts through impairment in the signaling pathway of PI3K-Akt, and Erk-MAP kinase resulting in mutagenesis, altered CYP 450 expression and consequent ROS accumulation thus exerting oxidative stress and DNA damage as described by Fig. 1. Tobacco smoke possess carcinogens derived from nitrosamines (NNAL, NNK & NNN), polycyclic aromatic hydrocarbons (PAHs), aromatic amines, volatile hydrocarbons, aldehydes, nitro compounds, phenols and inorganic composites (62, 63). Nitrosamines contained in tobacco substances are generated through a process known as nicotine and tobacco alkaloid nitrosation. Nitrosamines constitute a very high risk factor for sino-nasal cancers, nasopharyngeal cancers, and oral cavity cancers (22). During smoking process, nicotine reacts with nitrosamine to form NNN and NNK which are carcinogenic (25).

NNK mediates ROS induced DNA lesion and alteration of the microenvironment enabling tumor development. Elevated levels of ROS constantly activate transcription factors like NF-κB with consequent tumor progression (64). NNK found naturally in smoke from tobacco is pro-carcinogenic and naturally inactive but requires activation through metabolic processes to enable it exercise its carcinogenic potentials (65–68). CYPs converts NNK into metabolites that are DNA-reactive which can cause methylation, pyridyl-hydroxy-butylation and pyridyl-oxo-butylation in DNA nucleobases and consequent formation of DNA adducts (Fig. 3). NNK hydroxylation by α-Methylene produces methyl-diazonium ion and methane diazo-hydroxide that bonds with DNA to yield O4-methylthymine, O6-methylguanine (O6-mGua) and 7-N-methylguanine (7-mGua) (69). NNK α-Hydroxylation could occur at the methylene carbon or methyl carbon. α-hydroxy-methyl-NNK is produced when α-Hydroxylation reaction occur at the methyl carbon which could undergo glucuronidation to yield pyridyl-oxobutyl-diazohydroxide which subsequently act on the DNA to produce pyridyl-oxo-butylation (POB) adducts (70, 71).

NNN undergo three reaction types during its metabolism. Those reactions are; N-oxidation of pyridine, norcotinine formation and pyrrolidine ring hydroxylation (i.e α-hydroxylation at positions 2′and 5′, β-hydroxylation at positions 3′ and 4′) (72). α-hydroxylation of NNN at positions 2′and 5′ causes DNA adducts formation in the NNN pathway. The NNN α-hydroxylation reactions are catalyzed mainly by CYPs (73). The 2′-Hydroxy NNN spontaneously undergo ring unwinding to yield pyridyl-oxo-butyldiazo-hydroxide with identical structure to the one formed during NNK methyl hydroxylation. The 5′Hydroxylation yields electrophilic diazo-hydroxide which reacts with DNA to form adducts (65).

Nicotinic-acetylcholine receptors (nAChRs) comprises of five subunits with either homo pentamers or hetero pentamers which are responsible for ligand-gated ion channels formation in the plasma membranes

(74). Nicotine mimic acetylcholine to enable it bind to α subunit of the nAChRs (75). The affinity of the nicotine to α4β2 heteromeric nicotinic-acetylcholine receptors (α4β2nAChRs) is higher than the affinity of the nicotine to α7 homomeric nicotinic-acetylcholine receptors (α7nAChRs) (76). Smokers usually have increased biological activities of α7nAChR with impaired α4β2nAChR functions. Regrettably, α7nAChR is responsible for the regulation of cancer cell stimulating responses while α4β2nAChR is responsible for the regulation of cancer inhibitory activities thereby providing discriminatory support for cancer initiation and progression (77-81). nAChRs has functional diversity but functions mainly in cation (Ca2+) channels and contributes to regulation of various cellular activities in cell type specific pattern, reflecting different cellular cancer origins (82, 83). NNK interfere with the signaling of β-AdrR, stimulating growth and migration of the epithelial cells in the airway. Upregulated level of nAChR with simultaneous inhibition of α4β2nAChR in smokers alters the balance of the microenvironment thereby promoting α7nAChR activities, thus enhancing its activities on tumor cells (Fig.3) (84).

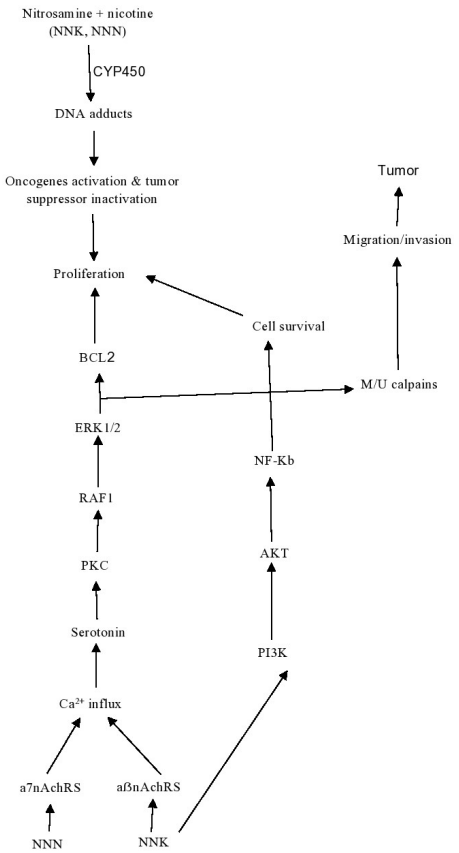

Figure 3: The possible toxicity from 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N′-nitrosonornicotine (NNN) exposures. NNK reacts with α7nAChR while NNN reacts with αβnAChRs to activate voltage gated-Ca2+ channel promoting Ca2+ influx into the cells and consequent depolarization of the membrane. This Ca2+ influx activates protein kinase C, serotonin, RAF1, extracellular signal-regulated kinases 1&2 (ERK 1 and ERK2), BCL2, causing proliferation. The pathway of Phosphatidylinositol 3-kinase (PI3K)-AKT plus NF-κB are also activated in response to the presence of NNK with resultant stimulation of proliferation and consequent inhibition of apoptosis

CONCLUSION

This study has deliberate about NPC risk from the precarious metabolic carcinogens such as acetaldehyde, nitrosamine and nicotine that could possibly derived from alcohols, salt preserved foods (diets) and tobacco smoking. Therefore, the best way to prevent and lower the risk of NPC is by reducing the exposure to the cancer promoting compounds by having a healthy diet and active lifestyle.

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