

Risk factors in larynx and oropharyngeal malignant neoplasms

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ABSTRACT

Alcohol consumption and cigarette smoking are most strongly and best-recognized risk factors of oral cavity cancer (OCC) and laryngeal cancer (LC). Alcohol multiplicative interaction includes acetaldehyde effects, reactive oxygen compound, epithelial cells irritation, increasing cross links penetration in mucosal cells. Some of the adverse effects of cigarette smoke and induction of oxidative stress by inhalation of free radicals of O₂ and N₂ are guanidine derivatives like polycyclic aromatic hydrocarbons (PAHs) and N-nitrosamines. There is substantial evidence that N-nitrosamines have an important role in DNA alkylation as a result of reactions with N, O and P atoms. The mutagenic properties of polycyclic aromatic hydrocarbons (PAHs) and N-Nitrosamines are known as important factors in pulmonary carcinogenesis. Alcohol consumption and cigarette smoking metabolism derivatives mutually play a role in carcinogenesis. Alcohol's role as solvent for polycyclic aromatic hydrocarbons (PAHs) and N-nitrosamines stimulates carcinogenesis absorption and transport to tissues. Alcohol has a role in the selective inhibition of nitrosamines metabolism in liver and cigarette smoking increased risk of extra hepatic tissues exposure of nitrosamines. These mechanisms are not mutually exclusive, but may play an important role as risk factor in extra hepatic carcinogenesis.

Keywords: head and neck cancer, risk factors, carcinogenic mechanisms, synergisms, alcohol tobacco synergism.

1. INTRODUCTION

The incidence of head and neck malignant neoplasms has reached in the recent years ca. 500,000 cases/year in America and Europe, according to the Italian Association for Cancer Research. Oropharynx malignant neoplasms account for about 1% of all malignant tumors and about 15% of the malignant neoplasms of the head and neck, and larynx malignant neoplasms for 0.8% of all tumors and 12% of the malignant neoplasms of the head and neck.

The most common risk factors for this type of malignant neoplasm are alcohol and tobacco abuse. Numerous epidemiological studies have confirmed that alcohol and tobacco interact in various ways, empowering each other's effect, and increasing the risk by more than 13 times compared with the risk calculated for each of the two factors [1].

2. ALCOHOL

Ethanol, a metabolite normally present in the body in small amounts (0.1 – 1mM), is the result of the fermentation produced by the gastrointestinal microbiota, or by metabolism in various tissues, especially the liver. A large part of the studies incriminate alcohol consumption as one of the most important epidemiological factors in the occurrence of malignant neoplasms (2-4% of all malignant neoplasms cases). Chronic alcohol consumption is considered the main risk factor for the hepatic, the upper airway and digestive tract carcinoma, and is suspected as a factor that increases the risk of breast malignant neoplasms [1]. After oral administration, ethanol is first absorbed in the blood in the oral cavity, and the pulmonary alveoli, respectively, in the case of ethanol vapors, and then it is evenly distributed in the tissues [2]. Approximately 20-30% of the ingested alcohol is absorbed by simple diffusion in the stomach, and the rest is absorbed in the duodenum, the small intestine and the colon [3]. A very small amount is metabolized in the stomach, small intestine, colon, kidney and lungs. Most of the alcohol absorbed (92-95%) is transported through the portal vein to the liver where the metabolism rate is the highest. A small portion of the ingested alcohol is eliminated in the urine, either in a nascent state or conjugated with glucuronic acid or ethyl glucuronide. Other ways of elimination of alcohol are excretion through breath or sweat. If

the relative risk (RR) for malignant neoplasms of the oropharynx for a daily consumption of 25 g of alcohol is 1, it increases to 1.5-2.05 for a consumption of 40 g of ethanol, and, for a daily consumption exceeding 100g, RR reaches 6.01 [4, 5].

Alcohol consumption increases the RR for malignant neoplasms of the larynx to a much lesser extent than for oropharynx malignant neoplasms [6]. In malignant neoplasms of the larynx, the main risk factor is smoking, 90% of these cancers being attributed to tobacco. In an epidemiological study on 750,000 individuals for the American Cancer Society, Bofetta and Garfinkel (1990) have found an increase in the risk of esophageal malignant neoplasms from 1.37 for a consumption of 12 g of alcohol/day to a risk of 5.8 corresponding to a consumption of 72 g/day. It is estimated that 25-68% of all malignant neoplasms can be attributed to alcohol consumption and more than 80% of them can be prevented by quitting alcohol and tobacco [7].

Multiple mechanisms are involved in the development of alcohol-related malignant neoplasms, including the effect of acetaldehyde, the first metabolite of ethanol oxidation, the oxidation processes catalyzed by oxidative enzymes belonging to the cytochrome P-450 system (monooxygenase microsomal system) that result in the generation of reactive oxygen radicals (ROI) and the activation of procarcinogens, potentially oncogenic

substances which do not produce mutational changes, but act on the mechanisms of regulation and control of the gene expression by promoting malignant transformation.

2.1. Hypothetical carcinogenic mechanisms of alcohol

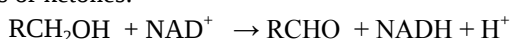
Several hypotheses have been proposed on how ethanol consumption increases the risk of malignant neoplasms [8]: i) Acetaldehyde is the first derivative of ethanol metabolism, incriminated in the development of malignant neoplasms. Ethanol is oxidized to acetaldehyde, a reaction catalyzed by alcohol dehydrogenase. Acetaldehyde is mutagenic in its ability to bind to DNA. Therefore, ethanol is co-carcinogenic, facilitating tumor initiation or acting as a promoter and less likely as a carcinogenic factor; ii) Alcohol can play an important role in tissues that are in direct contact, causing irritation of the epithelium and increasing the penetration of carcinogens in the mucosa, by solubilizing carcinogens, by increasing mucosal permeability, and by decreasing salivary secretion, resulting in an increased concentration of carcinogens; iii) Alcoholic beverages contain other co-carcinogens: nitrosamines, polycyclic aromatic hydrocarbons, mycotoxins [9]; Alcohol has a systemic immunosuppressive effect [10].

2.2. Local effects of alcohol

Alcohol acts as a solvent that increases the penetration of carcinogenic compounds in the mucosa. Ethanol can facilitate the absorption of carcinogens, in particular those found in cigarette smoke, through the solvent effect or by decreasing the permeability of the epithelial cell membrane. Proposed permeability decreasing mechanisms include the removal of the phospholipid bilayer structural lipids by their dissolution to ethanol, the redistribution of molecules in the epithelial cell membrane structure, or changes in the metabolism of lipids [11]. Chronic alcoholism causes parenchymal, parotid and submaxillary glands atrophy and metaplasia, which modifies salivary secretion by increasing viscosity. Thus, the oral mucosal surface under a lack of drainage will be exposed to higher carcinogen concentrations [12]. Other local mechanisms include the toxic effect of concentrated alcohol on the epithelium, impaired motility of the esophagus, and increase in the gastro-oesophageal reflux, which may result in epithelial metaplasia [13].

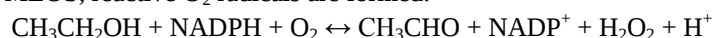
2.3. Acetaldehyde (AA)

Acetaldehyde, the first metabolic product of ethanol, results from the action of several types of enzymes: cytosolic alcohol dehydrogenase, alcohol oxidation microsomal system, and catalases. The enzyme family of alcohol dehydrogenase (ADH) consists of five classes comprising 20 isozymes. All are dimeric metalloenzymes with subunits of about 40 kDa, each subunit containing in the active site two zinc atoms bound to a cysteine residue. Alcohol is oxidized by alcohol dehydrogenases to aldehydes or ketones:



The family enzymes are found in the soluble fraction of the liver, kidney, lung, and probably most important in the metabolism of exogenous alcohols. The reaction goes to metabolize alcohol in the mitochondria. The oxidation of alcohol to aldehyde is considered as a response of the activation of toxicity (because aldehyde is toxic in relation to alcohol), and the second aldehyde oxidation reaction to acetic acid represents the detoxification step. Aldehyde cannot be excreted as it is lipophilic

and is covalently bound to proteins. All family enzymes include the electron acceptor coenzyme NAD^+ to oxidize ethanol to acetaldehyde [3]. Class I ADH is present in the liver and in the lower digestive tract, and classes II-V are found in the mucosa of the digestive tract, class IV being specific especially for the upper tract. The polymorphism of class I alcohol-dehydrogenases can modulate the AA resulting from the metabolism of ethanol. An increased risk of oropharyngeal and laryngeal malignant neoplasm was reported in homozygous individuals for the allele encoding enzymes more effective in producing AA ($ADH1C^*1$, $ADH1B^*2$), a homozygosity associated with a significantly higher level of AA in saliva [14, 8]. To a small extent, ethanol is also metabolized by the microsomal ethanol oxidizing system (MEOS), located in the rough endoplasmic reticulum of the epithelial cells of the digestive tract. Chronic alcohol consumption increases 10 to 20 times the activity of the secondary metabolic pathway involving, in particular, the cytochrome P-450E1 (CYP2E1). By activating MEOS, reactive O_2 radicals are formed:

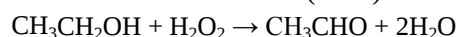


MEOS enzymes are also involved in the oxidation of xenobiotics, including procarcinogens (nitrosamines, aflatoxins, polycyclic hydrocarbons, hydrazines). The main function of the cytochrome is the detoxification of xenobiotics by reducing the molecular O_2 , to produce hydrophilic xenobiotics (which may be eliminated through urinary excretion) and ROI (O_2^- superoxide and hydrogen peroxide) having a cytotoxic effect when formed at a high rate, while antioxidant protecting cellular systems activity is poor. However, through the oxidation processes it catalyzes, the cytochrome activates procarcinogenic substances, transforming them into reactive compounds by acquiring epoxide electrophilic groups. The epoxide is a group of 3 atoms, two C and one O, covalently interacting with the negatively charged groups of the DNA, and causing point mutations [15].

Conjugation processes are subsequent to oxidation processes, and decrease the final concentration of the carcinogen. The process involves the intervention of second stage enzymes (epoxide hydrolase, glutathione-S-transferase, acetyltransferase, glucuronide-transferase, NADPH quinone reductase), distributed not only in the liver, but also in other organs.

In humans there is a genetic polymorphism of metabolism, reflected in a high individual variability of the activity of these enzymes. Therefore, only a certain proportion of individuals exposed to xenobiotics will develop a malignant neoplasm.

Catalases, located in peroxisomes, oxidize ethanol to acetaldehyde in the presence of hydrogen peroxide, playing a minor role in the metabolism of ethanol (< 2%).



In the gastrointestinal tract, AA may be generated from ethanol both by alcohol dehydrogenases activity in the mucosal cells, and by the activity of the microbiota in the oral cavity [16].

AA, as an alkylating agent, interferes with DNA synthesis and repair, and can thus lead to the emergence of malignant neoplasms. Many eukaryotic cell culture experiments carried out in vitro and in vivo have shown that AA has direct mutagenic and carcinogenic effects, resulting in point mutations in human lymphocytes, inducing the chromatin exchange between sister chromatids, and causing major chromosomal aberrations [17, 18]. AA inhibits O^6 -methylguanine transferase, an enzyme involved in

the repair of DNA damage caused by alkylating agents, starting from concentrations of 0.01 mM [19]. At concentrations between 1 and 5 mM, acetaldehyde activates oncogenes expression in oral keratinocytes.

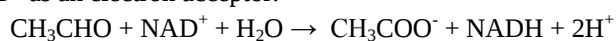
The best known (studied) change (N²-ethyl-2'-deoxyguanosine or N²-ethyl-dG) is produced by the reaction of acetaldehyde with a deoxynucleotide (dG). Fang and Vaca (1995) have found in the DNA from human lymphocytes from alcoholics a number of 2-3 lesions/10⁷ nucleotides, a significantly higher number than normal [20]. Terashima et al. (2001) showed that the presence of N²-ethyl-dG in DNA structure may generate transversions in vivo by the action of a DNA polymerase. [21]

The mechanism by which N²-ethyl-dG may determine the chromatin exchange between sister chromatids and the major chromosomal aberrations following exposure to AA is unknown. These effects emerge at AA concentrations of 40 to 1000 mol/l, concentrations determined in the saliva following alcohol consumption [22].

Recent studies have revealed that several other compounds may be formed by the reaction between AA and DNA. One of the most important is propano-dG (PdG), α -methyl- γ -hydroxy, N²-propano-2'-deoxyguanosine or Cr-PdG, which results from the interaction between crotonaldehyde (CRA) and dG or DNA. CrA is a mutagenic and genotoxic pollutant that is found in the environment, but which can also have an endogenous origin, probably from lipid peroxidation [23].

Sako et al. (2003) have shown that histones and amino acids (lysine or arginine) can facilitate the formation of Cr-PdG from AA and dG or from AA and DNA, requiring, however, AA concentrations in excess to those generated by the metabolism of ethanol. Theruvathu et al. (2005) have shown that polyamines at mM, having a role in cell growth, differentiation, regulation of synthesis of nucleic acids and cellular proliferation, can facilitate the formation of Cr-PdG from AA and dG or from AA and DNA, at physiological AA concentrations that may appear in the saliva after drinking alcohol. Cr-PdG formation could thus explain the hyperproliferation phenomenon, the acceleration of cell division in the oropharynx and esophagus [24].

Because of the mutagenic and cytotoxic effects, acetaldehyde is oxidized to acetate under the action of a family of enzymes, aldehyde dehydrogenases (ALDH), in the presence of NAD⁺ as an electron acceptor.



A secondary AA metabolism pathway is the aldehyde oxidation microsomal system, similar to the ethanol oxidation microsomal system. The third pathway of metabolism is catalyzed by aldehyde oxidase, the enzyme which catalyzes acetaldehyde oxidation in the presence of oxygen [3].

In humans, the alleles of ADH and ALDH induce higher levels of AA after alcohol consumption and increase the relative risk of malignant neoplasms in alcoholics.

The study on the mutant ALDH2 genotype and the occurrence of malignant neoplasms in Japanese alcoholics showed that the frequency of inactive ALDH2 is very high in alcoholics with cancer of the oral cavity, oropharynx, hypopharynx, larynx, esophagus, and colorectal cancer [25].

At the same, these people also show high levels of AA in the saliva due to the expression of alleles corresponding to ADH,

and thus releasing AA directly on the surface of the upper digestive tract mucosa [26]. Increased AA salivary level in these individuals, as in those who do not express ALDH enzyme activity, may explain the increased risk of malignant neoplasms, since AA in contact with the pharynx mucosa has an irritant effect and stimulates epithelial cell division rate [27].

The same changes have been observed in laboratory animals fed with alcohol for more than 6 months. Morphometric analyses in mice show increased nuclear volume of the basal layer cells of the oral cavity associated with an increase in the proportion of cells in S phase and a decrease in the thickness of the epithelium, indicating mucosal atrophy followed by hyperproliferation as a repair mechanism, but which increases the risk of malignant transformation [28, 29, 30].

Acetaldehyde also results from oral microbiota metabolism. Adult saliva contains 6×10^9 microorganisms/ml represented by Gram-positive organisms (Staphylococcus aureus, Staphylococcus epidermidis, Stomatococcus, Corynebacterium, Lactobacillus and 5 species of Streptococcus) and Gram-negative organisms (Neisseria, Veillonella, Candida albicans, bacteria of the genus Haemophilus or Bacteroides). A significant increase in the level of AA was detected in the saliva of healthy volunteers following the ingestion of a moderate dose of alcohol. Salivary acetaldehyde level was 10 to 20 times higher compared to the level of AA in the blood. Smoking quickly changes the microbiota of the oral cavity, Gram-negative microorganism being replaced by Gram-positive ones, which causes an increase in the concentration of AA by 50-60% to values in non-smokers [31].

2.4. Alcohol and DNA methylation

Changes in the degree of methylation of cytosine residues are frequent in human malignant neoplasms, but the relevance of this phenomenon as an epigenetic factor is not fully understood [32]. DNA methylation is crucial in the control of gene expression (hypermethylation has the effect of silencing genes, and hypomethylation can induce gene transcription rate increase). The hypermethylation of tumor growth suppressor genes and the hypomethylation of certain oncogenes may lead to cell differentiation and proliferation [33].

Hypomethylation may be due to lower levels of S-adenosyl-methionine, an important donor of methyl groups, and increased levels of S-adenosyl-homocysteine [34]. Chronic alcoholism interferes with the absorption of vitamin B involved in transmethylation reactions, which degrades the synthesis and transfer of methyl groups.

2.5. Alcohol and immune surveillance

Chronic alcohol consumption interferes with immune surveillance and is involved in the atrophy of the thymus and spleen, in the loss or redistribution of peripheral blood leukocytes, and in the decrease of the cell- and humoral mediated immune response [35]. Experimental and clinical studies have shown that chronic alcoholics are more susceptible to bacterial infections and malignant neoplasms [36]. Ethanol consumption reduces the number and the cytotoxic ability of NK cells, disrupting the circadian rhythm of granzyme, perforin and IFN- γ accumulation in the absence of which the cytotoxic activity of NK cells is decreased [37]. Alcohol consumption reduces the ability of neutrophils and monocytes to adhere and migrate through the vascular endothelium to the inflammatory focus, to phagocyte and

to lyse microorganisms. In a suspension of neutrophils/human monocyte cultures collected from healthy volunteers, acute administration of alcohol produces the inhibition of the phagocytic function of macrophages and reduces the expression of Fcγ receptors involved in phagocytosis [38]. Monocytes of alcoholic patients produce a smaller amount of inflammatory monokines TNFα, IL-1 and IL-6, which shows that ethanol can inhibit the inflammatory response by stimulating the bacteria [39].

3. TOBACCO

In Europe, the habit of smoking is relatively recent, growing rapidly in the first half of the twentieth century. The monograph "Tobacco Smoke and Involuntary Smoking" (IARC, 2004) concluded that smoking increases the risk of lung, nasopharyngeal, esophageal, gastric, hepatic, pancreatic and colon cancers, produces various malignant neoplasms of the oral cavity, leukemias, adenocarcinomas, squamous cell cancer, and the risk of malignant neoplasms is increased by alcohol consumption associated with smoking [41, 42, 43].

Smoking has been associated with malignant neoplasms of the larynx, oropharynx and hypopharynx, and is the leading cause of lung malignant neoplasms in non-smokers [41, 43, 44]. Nicotine itself is not carcinogenic, but each cigarette and cigarette smoke contain an association of carcinogens and co-carcinogens. Most tobacco carcinogens require metabolic activation. The disruption of the balance between detoxification and metabolic activation of various compounds in tobacco is the cause of malignant neoplasms occurrence [45].

Smoke results from incomplete tobacco burning. Burning is only complete on the lit end of the cigarette, where the temperature may exceed 900° C, determines the increase of the viscosity of the gas mixture, and, concomitantly, the increase of resistance to the passage of gas drawn through the cigarette. This effect forces the draw of air mainly from the edge of the coal, on the burning line of the paper, rather than directly from the coal. The depletion of O₂ during the combustion from the inside of the coal and the decrease of the air flow around it form an anaerobic area behind the coal, where, although the oxygen is lacking, the temperature is so high as to cause the thermal degradation of the unlit tobacco. The volatile and semivolatile constituents from cigarette smoke come from this pyrolysis-distillation area [46]. As the distance to the filter decreases, burning becomes incomplete, and the amount of harmful substances increases.

Cigarette smoke (environmental tobacco smoke – ETS, or "secondhand smoke" – SHS) is a combination of two types of smoke: smoke emitted by the lit portion of the cigarette (sidestream smoke – SS) and smoke exhaled/released by the smoker (mainstream smoke).

Cigarette smoke contains 10¹⁰ particles/ml with diameters of 0.1-1 μm dispersed in water vapor and other gases (nitrogen, carbon dioxide, carbon monoxide). The burn of a cigarette results in 2 liters of smoke containing about 4000 chemical compounds which are formed as a result of physicochemical and thermodynamic processes. Over 300 of these substances are toxic and more than 60 are carcinogenic.

The list of carcinogens in cigarette smoke (1-3 mg/cigarette) comprises polycyclic aromatic hydrocarbons formed by the incomplete combustion of the organic matter

In alcoholics, the number of T lymphocytes decreases due to increased thymocyte apoptosis rate [40]. The quantitative decrease of MHC II molecules on the surface of lymphocytes and monocytes exposed to alcohol is one of the causes of the inability of T-lymphocytes to be activated by antigens.

B lymphocyte dysfunctions are revealed by high levels of IgG and IgA in the serum, although the number of B lymphocytes is not altered [8].

(benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene), N-nitrosamines (N-nitroso-ethyl-methylamine, N-nitroso-dimethylamine, N-nitroso-diethyl-amine, N-nitroso-piperidine, N-nitroso-pyrrolidin, N-nitroso-diethanol-amine, N-nitroso-nornicotine = NNN, 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone = NNK), aromatic amines (2-toluidine, 2-nafthil-amine, 4-amino-biphenyl), aldehydes about 1 mg/cigarette (formaldehyde, acetaldehyde), other organic substances (benzene, catechol = 1,2-dihydroxy-benzene, 1,3-butadiene, isoprene, acrylonitrile), and inorganic compounds (nickel, chromium, cadmium, lead, and polonium-210) [47].

3.1. The pulmonary physiological effects of smoking

In smokers, pulmonary macrophages, including the alveolar ones, are activated by the irritating effect of tar and secrete proinflammatory ILs (IL-1, TNF, IL-6), with a positive chemotactic action for neutrophils, infiltrating the lung parenchyma. The local immune response is repressed due to the excess of free radicals. Smokers need 4 times more vitamin C/day to counteract the oxidative effects of free radicals. With the activation of macrophages, the inflammatory process develops and becomes permanent. Tobacco smoke causes bronchial mucous hypersecretion, alters ciliary movement, inhibits the function of alveolar macrophages, and causes the hypertrophy and hyperplasia of mucus-secreting glands, resulting in the obstruction of the drainage of bronchial secretions accumulated. Tar in cigarette smoke inhibits the activity of antiprotease enzymes (α₁-antitrypsin) and stimulates the release of proteolytic enzymes by polymorphonuclear leukocytes. Inhalation of cigarette smoke can cause a sharp increase of the airway resistance by smooth muscle contraction, vagally mediated, probably through the stimulation of the receptors in the submucosa. The removal of particles deposited in the airways is done differently in smokers versus non-smokers. In non-smokers, the removal begins immediately after inhalation and is fast for all particles deposited on the central airways, then slower but without retention for particles deposited on small and peripheral airways. In smokers, the purification phenomena are delayed and prolonged with a storage of foreign particles in the airways. Along with the direct toxicity of tobacco, in the airways, toxicity is enhanced by the inhibition of the activity of leukocytes that purify toxic substances.

3.2. The effects of polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons are metabolized in the liver, kidneys, lungs, are excreted in the urine, or stored in the adipose tissue. Through the cytochrome P450-catalyzed metabolism, PAHs are transformed into dihydrodiol-epoxide which covalently binds to DNA or produce oxidative stress through the generation of ROI. Since PAH molecules are

generally asymmetric, they can generate more isomers with different biological properties. Polycyclic hydrocarbon plane rings can be inserted between the bases making up the helix of DNA, causing its distortion, in particular by binding to the amino groups of adenine and guanine [48]. DNA-PAH compounds block the activity of polymerases, and interfere with DNA repair [49]. Repairers' failure and constant exposure to PAHs induces mutations especially in the structure of the p53 gene [50].

PAH binding to nitrogenous bases determines the segmenting of the macromolecule by loosening the bonds between the carbohydrate and the phosphate group, the depurination and subsequently the substitution of the purine base with pyrimidine one [51].

DNA-PAH bases pair erroneously. Pairing errors can be transitions, when a purine base is replaced by another purine base, or transversions, when a purine base is replaced by a pyrimidine base. For instance, during the replication of the O⁶ in methylguanine, instead of forming a pair with cytosine, it binds to thymine, producing the transversion CG → AT [52]. The guanine-thymine transversion is associated with activation of the c-Ha-ras proto-oncogene, while the adenine-thymine transversion is associated with activation of the ras proto-oncogene [53].

In the initiation stage of malignant neoplasm, the nucleotide altered by the interaction with the chemical carcinogens either has no effect on the cell being repaired, or produces a change that cannot be repaired, and induces the synthesis of a protein involved in the regulation of cell growth and division with an altered structure and function. Such a cell becomes a target for malignancies. Chemical carcinogens serve as initiators, and their effect is completed by other chemical agents, called promoters.

3.3. The effects of nicotine

Research has shown that nicotine alone is not mutagenic. In small doses, nicotine acts on the central cholinergic receptors in only 7-10 seconds following inhalation, and stimulates the effects of adrenaline release, with which it is structurally similar, both having a quaternary N atom. In large doses, nicotine is a depolarizes nicotinic receptors. In addition, it determines the increase in the level of dopamine, a mediator of the synaptic transmission in the CNS both by inhibition of monoamine oxidase, which is responsible for the metabolism of dopamine, as well as through the stimulation of dopamine release. Nicotine causes tachycardia, increased blood pressure, and has a hyperglycemic effect by blocking the release of insulin. It does not accumulate in the body due to the fact that it metabolizes quickly, with a half-life of about 2 hours.

3.4. The effects of nitrosamines

Nitrosamines, carcinogens in cigarette smoke, are generated mainly by pyrolysis, but may also have an endogenous origin by the nitrosation of nicotine metabolism products (cotinine, normicotine) [54]. Nitrosamines have a bioalkylant effect on DNA, by the reaction with N, O and P atoms. The term "bioalkylation" means the process by which an alkyl group (methyl, ethyl, propyl) binds directly to a C, O, N, S atom of organic compounds. They often work as acceptors of the group – CH₃ in primary and secondary metabolic processes. The process is called "biomethylation" and has major metabolic importance.

The most carcinogenic nitrosamines, N-nitroso-nornicotine

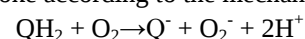
and 4-(N-nitrosomethylamino)1-(3-pyridyl)-1-butanone, generate DNA compounds, mainly O₆-methyl-guanine, which interferes with DNA replication [55]. These compounds generate transitions in all dividing cells, including those of the immune system. The metabolism of nitrosamines involves activation through oxidation catalyzed by cytochrome P450 enzymes, and then conjugation to DNA, catalyzed by the glutathione-S-transferase enzyme [56]. Normally, DNA-nitrosamine compounds are removed by DNA-repair mechanisms. If, however, these compounds remain during DNA replication, permanent mutations result [44].

Mutations in oncogenes or tumor growth suppressor genes generate cells bearing the distinctive mark of neoplastic cells: they are no longer controlled by the growth factors of the body, they can prevent apoptotic mechanisms and immune surveillance, they can prevent apoptotic mechanisms and immune surveillance, they may generate new blood vessels, can invade new tissue and can metastasize [47]. The cells with modified DNA can be removed by apoptosis. But, once activated, nitrosamines also trigger cascades of protein kinases (ERK1/2, PKCα, PI3K/Akt, MAPK) that have the effect of uncontrolled cell proliferation, decreased apoptosis rate, and tumorigenesis [57].

3.5. Other carcinogens

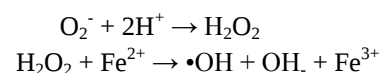
The tobacco plant absorbs and carries heavy metal and radioactive ions from the soil to the leaves, where they accumulate. The most abundant redox-inactive metals present in the leaves of the tobacco plant are cadmium, lead, mercury and arsenic, which inhibit the activity of antioxidant enzymes. Redox active metals (copper, iron, nickel and chromium) cause oxidative stress by generating ROI.

Heavy metals inhibit DNA repair processes [45]. Pryor (1983) associated the radicals in the tar produced by the burning of tobacco to hydroquinone and catechol (dihydroxybenzene), and suggested that these radicals can induce physiological changes associated with smoking. Semiquinone radicals (Q[•]) are concentrated in the aqueous extract of tobacco tar, and are derived following the autooxidation of hydroquinone (QH₂) and catechol in the cigarette smoke according to the mechanism:



Generally, quinone toxicity derives from ROI production by redox reactions and the formation of covalent bonds with essential biological molecules, especially those containing thiol groups.

Because of the disparity of superoxide radicals, peroxide radicals are generated spontaneously, which then, in the presence of metal ions, generate hydroxy radicals, very strong oxidizing agents.



The current concepts of ROI activity mechanisms aim two pathways: i) intracellular redox balance alterations. Compared to the extracellular medium, the cytoplasm is maintained in strong "reductive" conditions. These are accompanied by the "redox buffer" ability of intracellular thiols, glutathione (GSH) and thioredoxin (TRX). Both thiol systems oppose the intracellular oxidative stress by reducing H₂O₂, lipid superoxides and peroxides (reactions that are catalyzed by peroxidases); ii) oxidative modifications of proteins. ROIs can alter the structure and function of proteins by modifying amino acid residues, inducing

the dimerization of proteins, and interacting with other metal complexes (Fe-S).

The oxidative modifications of amino acids in the protein functional domain may involve multiple pathways: i) modification of proteins by the oxidation of cysteine residues. The sulfhydryl group of a cysteine residue can be oxidized to sulfenic (-SOH), sulfinic (-SO₂H), sulfonic (-SO₃H), or S-glutathionyl (-SSG) derivatives. If the cysteine residue is located in the catalytic site of the enzyme, its activity will be impaired, and, if it is located within the DNA binding site, it will affect the ability of transcription factors to bind to DNA; ii) the formation of intramolecular disulfide bonds by the oxidation of two or more of cysteine residues. This results in changes in the protein conformation which alter their activity; iii) protein dimerization by forming intermolecular sulfide bonds or cross-linking of two identical (homodimerization) or different (protein heterodimerization) protein tyrosine residues; iv) the active sites of metallo-enzymes often include Fe-S tetranuclear complexes (4Fe-4S) which are targeted by the superoxide radical. Fe regulatory proteins are susceptible to oxidation which does not directly influence their ability to bind to mRNA, and therefore does not alter the translation of, but marks them for the binding to ubiquitin and, consequently, for the degradation to proteasomes [58], v) Tobacco smoke is the main source of exposure to formaldehyde, a group I carcinogen according to the International Agency for Research on Cancer (IARC) classification, having a concentration three times higher than normal (5-7 µg/g dry weight in snuff tobacco). Formaldehyde is also formed endogenously, having concentration of 2 to 3 mg/l in human blood [59].

Inhalation of formaldehyde is due to the formation of DNA-protein bonds (protein-NH-CH₂-NH-DNA) in the respiratory mucosa which disrupts DNA replication and transcription, can cause breakage of DNA strands, numerical chromosomal aberrations, sister chromatid exchange of chromatin, increase in micronuclei, changes in cell growth and differentiation, and a higher incidence of hyperplasia, metaplasia and dysplasia. It was demonstrated that the inhalation of formaldehyde produces nasal tumors in laboratory animals, 50% of them having a point mutation in the p53 tumor growth suppressor gene. Co-administration of formaldehyde with known carcinogens has amplified the effects of each carcinogen administered separately. Formaldehyde is a weak mutagen, and, therefore, may only be a starter. On the other hand, it causes damage to the tissue with which it comes into contact (burns, cellular degeneration, necrosis and subsequent inflammation, squamous cell metaplasia, and epithelial hyperplasia), and results in compensation, cell proliferation, particularly in the areas of transition from a type of epithelium to another, which contributes significantly to the occurrence of malignant neoplasms [60].

4. CONCLUSIONS

Alcohol is the main risk factor for liver, upper airway, and digestive tract carcinoma, acting through multiple mechanisms, including the effect of acetaldehyde, generation of reactive oxygen radicals, activation of procarcinogens, epithelial irritation and increased penetration of carcinogens in the mucosa, or through its

3.6. The combined effect of alcohol and tobacco

Numerous epidemiological studies have confirmed that alcohol and tobacco mutually reinforce their effects [7, 61, 62, 63, 64, 65]. Tuyns (1978) demonstrated that the use of more than 80 g/day of alcohol increases the relative risk (RR) of esophageal cancer by a factor of 18. At the same time, only the smoking of more than 20 cigarettes/day increased the relative risk to 5. Acting together, alcohol and tobacco can increase the risk by a factor of 44 [8]. The latest IARC research reported an increase of RR from 5 to 77.3, the tremendous variation being caused by the different amounts of ethanol and tobacco consumed: for tobacco from 10 g/week to 280 g/week, and for alcohol from a consumption of 20 g ethanol/day to about 140 g/day [41].

In smokers consuming ethanol, AA formed in the mouth by the oxidation of ethanol under the influence of the enzymatic activity of bacterial ADH continues to form endogenously even after several weeks of withdrawal from tobacco, the level of acetaldehyde being 2 times greater in smokers exposed to ethanol even if they had not smoked during this exposure as compared to its level in non-smokers who had used the same amount of alcohol [66, 67, 68, 69, 70]. The cause of the increase of the concentration of acetaldehyde in smokers exposed to ethanol is the changing of the normal oral microbiota composed mainly of aerobic bacteria that produce large amounts of AA (*Streptococcus salivarius*, *S. viridans*, *Corynebacterium* sp., *Stomatococcus* sp.) [71].

The activation of CYP2E1 in the upper digestive tract may be particularly relevant, taking into account the synergistic effect of alcohol and smoking, and the cytochrome role in the activation of cigarette smoke procarcinogens (nitroso-pyrrolidine formed by burning tobacco leaves infected by nitrites). The interaction between ethanol and procarcinogen metabolism is complex, and may be dependent on the degree of activation of CYP2E1, the procarcinogens' chemical structure, and the presence or absence of ethanol during procarcinogen metabolism.

Simultaneous administration of ethanol and nitrosamines in experimental studies led to the development of extrahepatic tumors. The mechanism consists in the inhibition of the metabolism of nitrosamines in the liver caused by alcohol, followed by extrahepatic tissues increased exposure to nitrosamines. Measurement of dimethyl-nitrosamine metabolism radioactively marked (DMN) in the liver tissue and esophageal epithelium suggests that alkylation of esophageal DNA may be the result of the inhibition of DMN metabolism in the liver [72]. Furthermore, the administration of ethanol also causes the increase of O⁶-methyl-guanine derived from DMN in the DNA from the upper digestive tract mucosa [73, 74]. Following administration of the carcinogen N-nitroso-methyl-benzyl-amine in the esophagus in monkeys followed by water with 20% alcohol, the level of O⁶-methyl-deoxiguanosine in the esophagus was increased three times.

immunosuppressant systemic effect. Acetaldehyde, the primary product of the oxidation of ethyl alcohol is a major neoplastic risk factor, having mutagenic and carcinogenic direct effects caused by its interference with DNA synthesis and repair. Polymorphisms and mutations in genes encoding the enzymes that metabolize

ethanol and the genes encoding the enzymes that metabolize acetaldehyde increase the risk of malignant neoplasms by increasing AA concentration in the saliva. Chronic alcohol consumption generates ROI, activates *procarcinogenic* substances that, by making covalent bonds with DNA, induce the appearance of point mutations, *generates changes in the degree of methylation of tumor growth suppressor genes and certain oncogenes*, resulting in the increased rate of cell division, and *interferes with the immune surveillance* by reducing the number and cytotoxic ability of NK cells, reducing the capacity of phagocytosis and lysis neutrophils and monocytes, increasing the apoptosis rate of thymocytes, and reducing the capacity of differentiation and proliferation of T lymphocytes. IARC studies concluded that smoking is a neoplastic risk factor. Effects range from hypertrophy and hyperplasia of mucus-secreting glands, prevention of drainage of the bronchial secretions, inhibition of

alveolar macrophages, to the formation of DNA-nitrosamines compounds, nitrogenous base pairing errors, activation of protein kinase cascades that have the effect of uncontrolled cell proliferation, decreased rate of apoptosis and tumorigenesis, inhibition of DNA repair processes, alteration of intracellular redox state, or the occurrence of oxidative changes in the protein structure. Alcohol and tobacco interact in various ways, mutually empowering their effect. Alcohol serves as a solvent also for carcinogens in tobacco smoke, inhibits the metabolism of nitrosamines in the liver, and, therefore, increased the exposure of extrahepatic tissues to nitrosamines in tobacco. In ethanolic smokers, normal oral microbiota change by replacing Gram negative bacteria with Gram positive bacteria and poor oral hygiene increases salivary acetaldehyde concentration, the main neoplastic risk factor.

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