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Toxicological Profile of NNK, Carbon Monoxide and Benzo[a]pyrene

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Abstract Tobacco smoke contains a complex mixture of over 5000 toxic substances, of which at least 69 are carcinogenic, being also involved in other pathologies such as cardiovascular and pulmonary diseases.
The purpose of this review is to highlight the toxicological profile of some of the most harmful xenobiotics in tobacco smoke: NNK (tobacco-specific nitrosamine - Nicotine-derived nitrosamine ketone), benzo[a]pyrene and carbon monoxide.

Keywords tobacco, NNK, benzo[a]pyrene, cancer, carbon monoxide

1. Introduction

Tobacco is the main cause of cancers that can be prevented. WHO (World Health Organization) estimated approximately 1.27 billion tobacco users globally.
Only tobacco consumption accounts for about 5.4 million deaths per year, and it is estimated that the number of deaths in this century will reach one billion if the global tobacco consumption rate remains at the current level [1].
The overall rates of deaths from cancer are twice as high for smokers as for non-smokers. Hardcore smokers have a four times higher risk of death than non-smokers [2].
The most common type of cancer caused by tobacco consumption is lung cancer. Cigarette smoking is the cause of approximately 80 to 90% of all cases of lung cancer, being the leading cause of cancer death in both men and women, accounting for around 80% of the deaths caused by this disease [3].
The risk of cancer is five to ten times higher for smokers than for non-smokers [4].
Smoking also increases the risk of cancer of the oral cavity, pharynx, larynx, esophagus, stomach, pancreas, cervix, as well as of the kidney or the bladder [5].
Apart from cancer, smoking also increases the risk of developing lung diseases, such as chronic bronchitis and emphysema, and has been found to worsen asthma symptoms in adults and children. Cigarette smoking is the most significant risk factor for chronic obstructive pulmonary disease (COPD) [6].
Also, smoking substantially increases the risk of cardiovascular disease, including stroke, myocardial infarction and aneurysm [7,8]. Cardiovascular disease accounts for about 40% of all smoking-related deaths [9]. Smoking increases

the risk of coronary artery disease, the leading cause of death in the United States. Smoking may increase the risk of developing diabetes, rheumatoid arthritis and immune disorders [5].

Cigarette smoke is a complex and dynamic reagent mixture of over 5000 toxic substances, of which at least 69 are carcinogenic [5].

In this article, we focused on the toxicological profile of three toxic xenobiotics in cigarette smoke, namely: NNK (tobacco specific nitrosamine), carbon monoxide and benzo[a]pyrene.

2. NNK

NNK is a tobacco-specific nitrosamine. The name according to IUPAC (International Union of Pure and Applied Chemistry) is 4- (methylnitrosamino) -1- (3-pyridyl) -1-butanone.

It is potent for humans and animals. Studies have shown that it increases the risk of cancer [10].

The chemical structure of NNK is shown in Figure 1.

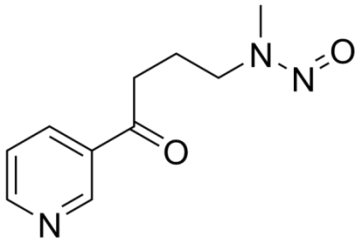

Figure 1: The chemical structure of NNK

Toxicokinetics

Absorption and Distribution

Absorption of NNK in smokeless tobacco users has been demonstrated by the detection of its metabolites 4- (methylnitrosamine) -1- (3-pyridyl) -1-butanol (NNAL) and NNAL-glucuronide (NNAL-N-Gluc) in the urine and plasma and of NNAL-N-oxide in the urine. Similar results were obtained for smokers [11].

Metabolization

NNK and NNAL require metabolic activation to express carcinogenicity. Cytochrome P_{450}-catalyzed alpha-hydroxylation in the NNK or NNAL methyl group generates reactive intermediates that alkylate the DNA and form DNA-pyridyloxobutyl (POB) adducts or DNA-pyridylhydroxybutyl (PHB) adducts. NNK is metabolised to NNAL in a reversible and stereoselective manner, and the specific tissue retention of (S) -NNAL is considered to be important for NNK carcinogenicity [12, 13].

Zhang et al. examined the formation of POB-DNA and PHB-DNA adducts in extra-hepatic tissues of F344 rats receiving chronic NNK and (R) and (S) -NNAL (10 ppm in drinking water for 1-20 weeks). POB-DNA and PHB-DNA adducts were quantified in the olfactory mucosa, nasal respiratory mucosa, oral mucosa and pancreas of the treated rats.

The adduct formation in the nasal respiratory mucosa exceeded that of the other tissues. The major adduct was 0 (2) - [4- (3-pyridyl) -4-oxo-1-butyl] thymidine, (0 (2) -POB- dThd) hydroxy-1-butyl-thymidine (0 (2) -PHB-dThd), followed by 7- [4- (3-pyridyl) -4-oxo-1-butylguanine] or 7- [4- (3-pyridyl) -4- hydroxy-1 -butyl] guanine 7-PHB-Gua). There was a remarkable similarity in the adduct formation between NNK and (S) -N-NAL groups, both of which being distinctly different from those in the (R) -N-NAL group [14].

For example, in the olfactory mucosa, concentrations of the POB-DNA adduct in the NNK and (S) -NNAL groups were not significantly different from each other, whereas the (R) -NALAL administration produced 6-33-fold less of POB-DNA adducts than administration of NNK. However, administration of (R) -NALAL produced significantly greater concentrations of PHB-DNA adducts than administration of NNK or (S) -NNAL. Similar trends have also been observed in the nasal respiratory mucosa, oral mucosa, and pancreas. These results suggest extensive retention of (S) -NNAL in various tissues from NNK-treated rats and support a mechanism in which preferential metabolism

of NNK to (S) -NNAL followed by sequestration of (S) -N-NAL in target tissues and reoxidation at NNK, is important for NNK tumorigenesis [14,15].

Elimination

Urine is the only way to excrete NNK metabolites for which data from studies on human subjects are currently available. Studies on laboratory animals indicate that urine is the primary route of excretion for NNK metabolites [11].

Toxicodynamics

Nicotine and nitrosamines form a devastating and fatal alliance. Nicotine activates the reward system of the brain, causing the desire to consume tobacco continuously, and is often accompanied by carcinogens that induce tumor initiation and progression [16].

NNK has a strong carcinogenic activity in vitro and in vivo and has been shown to contribute to several stages in the tumorigenesis of lung cancer [17].

Although many tobacco xenobiotics play an important role in the development of cancerous tumors, the strong effects of NNK and N'-nitrosonornicotine (NNN) are unique. Metabolically activated NNK and NNN induce harmful mutations in oncogenic and tumor suppressor genes by the formation of DNA adducts that initiate the onset of tumors. Moreover, NNK and NNN binding of acetylcholine nicotinic receptors promote tumor growth by enhancing and disrupting cell proliferation, migration and invasion, thereby creating conditions conducive to tumor growth. These two unique aspects of NNK and NNN synergistically induce cancers in individuals exposed to tobacco [18].

Metabolic activation of NNK and NNAL in adducts with DNA is critical for the expression of their carcinogenic activities. The process of metabolic activation has been extensively documented in laboratory animals. Cytochrome P_{450} enzymes are the main catalysts for this process, and those in the 2A family seem to be most effective in both humans and laboratory animals. NNK is a genotoxic compound. It has been shown to be mutagenic in bacteria, rodent fibroblasts and in human lymphoblasts in vitro. In animal studies, NNK caused cytogenetic effects in a variety of mammalian cells in vitro and induced a transformation of hamster pancreatic duct cells. In one single study NNAL was reported to be a mutagen in Salmonella. In addition to the classical carcinogenesis mechanisms that are undergoing adduct formation with DNA, NNK also binds to nicotinic receptors and other receptors, resulting in cascading effects that contribute to the development of cancer. These effects have been observed in experimental systems, including pancreatic and pulmonary cells from humans and laboratory animals [11].

3. Carbon Monoxide

Carbon monoxideis a highly toxic, odorless, insipid gas that results from the incomplete combustion of carbon/fuel. Inhalation causes damage to the central nervous system and asphyxia [19].

Toxicokinetics

Absorption

It is absorbed into the body by means of inhalation [20].

Carriage of carbon monoxide from the respiratory pathway to the erythroid cells is predominantly controlled by physical processes. Carbon monoxide transfer to hemoglobin binding sites is carried out in two sequential steps: transfer of carbon monoxide in the gas phase, between the airway and the alveoli, and the "liquid" phase transfer at the air-blood interface, including the haematid. Although the mechanical action of the respiratory system and molecular diffusion inside the alveoli are the key mechanisms of the gas phase transport, the carbon monoxide diffusion through the alveolar capillary barrier, plasma and the liver is the real mechanism in the liquid phase. [21]

Distribution

Carbon monoxide easily crosses the placental barrier [22].

Myoglobin is involved in the transport of oxygen from capillaries to mitochondriae in the muscle. Binding of carbon monoxide to the myoglobin in the heart and in the striated muscles in vivo was demonstrated for carboxyhemoglobin concentrations below 2% in the heart and 1% in skeletal muscles. It has been found that the carboxymyoglobin/carboxyhemoglobin saturation ratio is approximately 1 in the cardiac muscle and less than 1 in the skeletal muscle. These ratios did not increase with carboxyhemoglobin growth to 50% saturation [21].

The main factors that determine the final concentration of carboxyhemoglobin in the blood are: the amount of inspired carbon monoxide, alveolar ventilation at rest and during physical exercise, production of endogenous carbon monoxide, blood volume, barometric pressure, and relative lung diffusion capacity. The rate of alveolar diffusion and the binding of carbon monoxide to hemoglobin in the blood are the stages limiting the rate of absorption into the blood [23].

Metabolization

Carboxyhemoglobin is completely dissociable and, once acute exposure ceases, carbon monoxide will be excreted pulmonary. Only a very small amount is oxidized to carbon dioxide [22].

Elimination

Carbon monoxide is not a cumulative toxic in the true sense of the word. Carboxyhemoglobin is completely dissociable and once the exposure ceases, the carboxyhemoglobin will convert to oxyhemoglobin. The released carbon monoxide is removed at the pulmonary level [24].

The plasma half-life of carbon monoxide under normal conditions of recovery through air intake exhibits significant interindividual variation. For example, when the carboxyhemoglobin concentration is about 2-10%, the half-life varies from 3 to 6 hours [21].

Toxicodynamics

Carbon monoxide reacts with hemoglobin to form carboxyhemoglobin, a non-respiratory pigment. For example, exposure to air containing 0.4% carbon monoxide for 20-30 minutes leads to the conversion of 70% of the hemoglobin in the blood to carboxyhemoglobin [25].

The binding of carbon monoxide to hemoglobin to form carboxyhemoglobin and decreased capacity of oxygen carrying through blood, seems to be the main mechanism of action underlying the induction of toxic effects following exposure to carbon monoxide in low concentrations. The precise mechanisms by which the toxic effects induced by the formation of carboxyhemoglobin are not fully understood but probably include the induction of hypoxic conditions in many tissues of various organ systems. Various hypothetical mechanisms have been issued, alternative or secondary to carbon monoxide induced toxicity (in addition to the formation of carboxyhemoglobin), but it has not been demonstrated that they operate at relatively low carbon monoxide exposure levels (nearly ambient) [21].

Carbon monoxide binds through a strong bond to the hemoglobin ferric ion, reducing the release of oxygen into the tissues. Although this has been considered for many years to be the unique mechanism of toxicity of carbon monoxide, there is evidence to suggest that carbon monoxide also binds cytochrome a and a3 [24].

The affinity of hemoglobin for carbon monoxide is between 210 and 300 times higher than its oxygen affinity, the exact factor depending on the pH of the blood and the partial pressure of carbon dioxide. In addition, the presence of carboxyhemoglobin modulates the dissociation of oxyhemoglobin so that the remaining oxyhemoglobin is somewhat less effective in oxygen transport [25].

A unique feature of exposure to carbon monoxide is therefore that the level of carboxyhemoglobin in the blood is a biologically useful marker of the dose the individual has inhaled. Carboxyhemoglobin formation is a reversible process [21].

Symptoms of poisoning

Symptoms and signs of acute carbon monoxide poisoning correlate to a small extent with the concentration of carboxyhemoglobin measured at the time of hospital arrival [21,26].

The neurological symptoms of carbon monoxide poisoning include headache, dizziness, weakness, nausea, confusion, disorientation and visual disturbances. Exertional dyspnea, tachycardia, tachypnea and syncope are observed at continuous exposure [27].

When the carboxyhemoglobin concentration in the blood exceeds the 50% threshold, seizures and cardiopulmonary arrest may occur [28].

Frequent complications of acute carbon monoxide poisoning are myocardial damage, hypotension, arrhythmias and pulmonary edema. Perhaps the most insidious effect of carbon monoxide poisoning is the neuropsychological damage and neuro-behavioral consequences, especially in children. Carbon monoxide poisoning during pregnancy results in an increased risk for the mother by increasing the rate of short-term complications and for the fetus causing fetal death, developmental disorders and brain anoxic lesions.

Carbon monoxide poisoning occurs frequently, has serious consequences, including immediate death, involves complications and late sequelae, and is often overlooked [21].

Treatment of acute poisoning with carbon monoxide

The treatment includes administration of 100% oxygen and, in severe cases, hyperbaric oxygen. The half-life of carboxyhemoglobin is 6 hours in the normal air, 1.5 hours with administration of 100% oxygen and 23 minutes with administration of hyperbaric oxygen (at 3 atm pressure) [26,29].

4. Benzo[a]pyrene

Benzo[a]pyrene is a strong mutagen and carcinogenic agent. This xenobiotic stands for a public health problem due to its possible effects on industrial workers, as an environmental pollutant, and also as a component of tobacco smoke [30].

Benzo[a]pyrene (3,4-benzpyrene) is a polynuclear aromatic hydrocarbon composed of five condensed benzene cycles and formed during the incomplete combustion of organic matter. 3,4-Benzpyrene is mainly found in exhaust gases, cigarette smoke, coal tar, grilled foods, carbohydrate pyrolysis products, soot smoke, creosote oil, asphalt oils and shale. This substance is only used for research purposes. It is a carcinogenic substance for humans [31].

Chemical structure

The chemical structure of benzo[a]pyrene is presented in fig. 2.

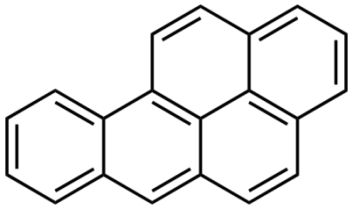

Figure 2: The chemical structure of benzo[a]pyrene

Toxicokinetics

Absorption

It is easily absorbed from the gastrointestinal tract and tends to localize mainly in the adipose tissue and fatty tissues such as breast tissue [32].

Distribution

The disappearance of benzo[a]pyrene from rat blood and rat liver after a single intravenous administration is very rapid, having a half-life in the blood of less than 5 minutes and a half-life of 10 minutes in the liver. In the blood and

liver, the initial rapid elimination phase is followed by a slower phase of elimination lasting 6 hours or more. A rapid balance is established between the benzo[a]pyrene in the blood and the one in liver, and its rapid disappearance from the blood is due to its metabolism and distribution into tissues [32].
According to Heredia-Ortiz et al., the critical determinants of benzo[a]pyrene kinetics are:

- hepatic metabolism of benzo[a]pyrene and rate of elimination of 3-hydroxy-benzo[a]pyrene as the most sensitive parameters;
- larger distribution of benzo[a]pyrene in the lungs compared to other tissues, followed by adipose and hepatic tissues;
- the large distribution of 3-OH benzo[a]pyrene in the kidneys;
- narrow tissue transfers of benzo[a]pyrene in the lungs and 3-hydroxy-benzo[a]pyrene in the lungs, adipose tissue and kidneys;
- greater participation in the enterohepatic circuit of 3-hydroxybenzo[a]pyrene [33].

Metabolization
Benzo[a]pyrene is metabolized to about 20 primary and secondary oxidized metabolites and a variety of conjugates. Several metabolites can induce mutations, transform cells and/or bind to cellular macromolecules. However, only its 7,8-diol-9,10-epoxide is currently considered to be a last carcinogenic metabolite [11].

Toxicodynamics
Benzo[a]pyrene is an ubiquitous environmental pollutant that can contribute to the development of cancer in humans. The most important carcinogenic metabolite of benzo[a]pyrene produced by cytochrome P_{450} (CYP) enzymes such as CYP1A1 and CYP1B1 is anti-benzo[a]pyrene-7,8-diol9,10-epoxide and binds covalently producing DNA mutations [34].
The genotoxic mechanism of action of benzo[a]pyrene involves metabolization to highly reactive species that form covalent DNA adducts. These anti-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adducts induce mutations into the K-RAS oncogene and tumor suppressor TP53 gene in human lung tumors and in corresponding genes in mouse tumors. Exposure to benzo[a]pyrene and complex mixtures containing benzo[a]pyrene also induce other genotoxic effects, all of which contribute to carcinogenic effects of benzo[a]pyrene in exposed human subjects [11].

5. Conclusions

Tobacco consumption is the main cause of preventable cancers, representing a major risk factor for lung cancer in particular, but also for other types of cancer.
The addictive potential of tobacco makes smoking cessation more difficult, thus increasing the risk of cancer, cardiovascular and lung diseases in consumers.
Unfortunately, the effect of smoking on human health is not limited to smokers, but it is also present in non-smokers exposed to environmental tobacco.
Tobacco smoke contains over 5000 toxic xenobiotics, of which at least 69 carcinogenic toxic substances, whose toxic action mechanism consists in the formation of DNA adducts of the respective xenobiotics or their electrophilic metabolites. Some of the adducts so formed are not detoxified by the body and induce permanent mutations in the cellular DNA sequence, which translates into uncontrolled cell proliferation and the appearance of cancer.
There is a need for a better understanding of the specific mechanisms by which tobacco induces carcinogenesis, as this would favor the discovery of new biomarkers, the development of more sensitive methods for the quantitative identification of tobacco-specific carcinogens, increasing the effectiveness of epidemiological studies and guiding the evolution of public health and politics health sector towards the implementation of improved approaches to prevent tobacco-related cancers.
It is also necessary to continuously inform users about the risk they are facing and to develop new smoking cessation adjuvant methods.

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