DEOXYRIBONUCLEIC ACID INTERACTIONS SPECIFIC TO BIOCHEMICAL INJURY INDUCED BY SOME CARCINOGENIC XENOBIOTICS

ZENO GÂRBAN[1,2], NICOLAE MANOLESCU[3,4], GHEORGHE DĂRĂBUŞ[5], ADINA AVACOVICI[2],FLORIN MUSELIN[5], JELENA SAVICI[5], ROBERT UJHELYI[2,6], SERGIU MICLOŞONI[5]

1. University of Agricultural Sciences and Veterinary Medicine of Banat "King Michael I of Romania" Timişoara, Faculty of Food Products Technology, Romania
2. Working Group for Xenobiochemistry, Romanian Academy-Branch Timişoara,
3. University of Agronomic Sciences and Veterinary Medicine Bucharest, Institute of Comparative Medicine, Romania
4. "Al. Trestioreanu" Institute of Oncology, Bucharest, Romania
5. University of Agricultural Sciences and Veterinary Medicine of Banat "King Michael I of Romania" Timişoara, Faculty of Veterinary Medicine, Romania
6. S.C. CaliVita International, Medical Department, Timişoara, Romania

zeno.garban@yahoo.com

Abstract: *At the origin of the neoplastic processes in which deoxyribonucleic acid is involved, one can mention as trigger factors a great number of xenobiotics which are present in the air, feedstuffs, foodstuffs and water having harmful effects on animals and humans. Xenobiotics of various sources : chemical (organic and inorganic compounds), physical (ionizing radiations, UV radiation) and biological (oncoviruses) can become carcinogenic agents.Submitted as a review the aim of this paper is to give data on the molecular mechanisms inducing biochemical injuries as a consequence of the interaction of deoxyribonucleic acid (DNA) with various chemical compounds. The interactions of DNA with the following organic compunds are discussed : aflatoxins (AF), polycyclic aromatic hydrocarbons (PAH), aromatic amines and the following inorganic compounds : some metal ions (M^{n+}) with toxicogene potential; nitrates (NO_3^-) and nitrites (NO_2^-).These interactions are of interest for comparative medicine due to their pathobiochemical and pathophysiological aspects. All the above mentioned adducts are frequently discussed in pathology being at the origin of the biochemical injury which can evolve to a carcinogen process. Initially the nuclear DNA is damaged and further on the cell. If the pathological process develops the result is the formation and growth of a neoplasm.*

Key words*: deoxyribonucleic acid, biochemical injury, xenobiotics*

INTRODUCTION

Deoxyribonucleic acid (DNA) is a macromolecule that contains the genetic information which controls the biological development of all cellular forms of life. In the environment (air, water, foods) there are numerous substances considered as chemical xenobiotics which can interact with DNA, altering its structure, and giving rise to adduct genesis. As a result of such interaction mutagen, carcinogen and teratogen processes can be triggered (Knudsen, 1989; Ames et al., 1993; Nassar, 2010).

Such chemical xenobiotics (organic or inorganic) can get into the organism by food along with nutrients and water intake as well as by air breathing or skin contact (Arcos et al., 1996; Kohlmeier, 2003; Creppy et al., 2005; Gârban, 2007). Among the organic chemical xenobiotics the most discussed for their carcinogenic effects are those with cyclic structure,

like: polycyclic aromatic hydrocarbons (PAH), mycotoxins, monomethyl aminoazobenzene (MAB), steroidic compounds (Bannett and Klich, 2003; Manolescu, 2003; Luck, 2005) and organic pesticides (Brooks and Roberts, 1999). All the chemical xenobiotics mentioned before give rise to adducts with DNA, e.g.: the benzo(a)anthracene, a well-known PAH, by binding to the adenine of DNA forms an adduct of type DNA-B(a)P ; MAB forms with DNA the adduct DNA-MAB; aflatoxins, especially AFB_1 , by binding to guanine forms the DNA-AFB_1 type adduct. Regarding the inorganic chemical xenobiotics it is known that potentially toxicogen metal ions like Cd^{2+}, Hg^{2+}, Sn^{2+} can bind to DNA giving rise to adducts of the DNA-M^{n+} type while nitrates converted into nitrites generate (in vivo) nitrozamines which further on form intermediate compounds and finally one of them alkylate to DNA.

In the present review issues related to pesticides, compounds of great importance in xenobiochemistry, are not approached because of their very extended domain. Data concerning organochlorine and organophosphorus compounds as well as carbamate and thiocarbamate will be exposed in a next work dealing with their interactions and biomedical effects. Knowledge on the structural peculiarities of DNA can allow a better understanding of the appearance of DNA adducts and carcinogenesis.

1. Structural characteristics of DNA - general aspects

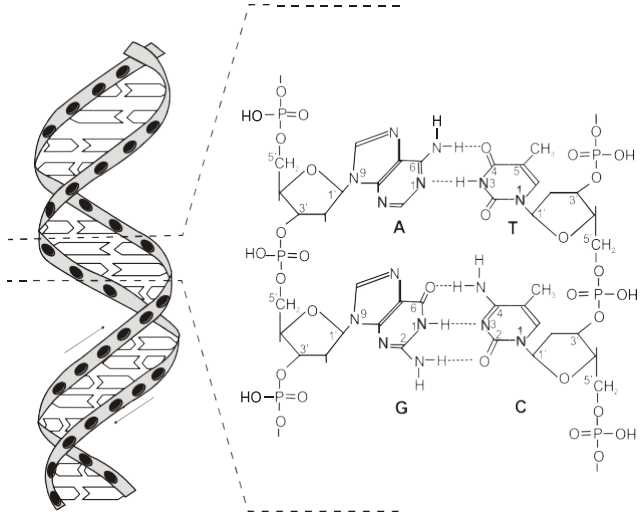


Fig. 1. DNA macromolecule (structural formula)

DNA is a macromolecular biopolymer ubiquitously spread in the animal and vegetal reign and constitute the genetic material of micro- and macroorganisms. This macromolecule is composed of building blocks called nucleotides. Each nucleotide consists of deoxyribose, a phosphodiesteric group and one of four nitrogen containing bases – adenine (A), thymine (T), guanine (G) and cytosine (C). Phosphates and deoxyribose of the adjacent nucleotide link to form a monostrand. In the DNA macromolecule there are two strands (fig.1) .

The nucleobases from one strand bind to the nucleobases from another opposite strand to form a double-stranded structure. The binding takes places according to the Watson

– Crick model: adenine forms two hydrogen bonds with thymine on the opposite strand, and guanine forms three hydrogen bonds with cytosine on the opposite strand.

2. Chemical injury of DNA and biogenesis of adducts

Experimental investigations initiated in 20th century regarding the interaction between DNA and small molecules of chemical xenobiotics revealed the appearance of some biologically non-compatible structures that, initially were named "molecular complexes". This denomination was considered inadequate and, later on, due to years of physical-chemical investigations and mechanic-quantum calculations over the electronic density they were named "molecular associations". Studying more thoroughly the donor-acceptor relationship between DNA and small molecules, the term "adducts" was preferred (Phillips, 2005). One of the major challenges in biochemical pathology (pathobiochemistry) was to define the structural and chemical basis for carcinogenesis and mutagenesis induced by DNA injuries (Wassom, 1980; Ames et al., 1993).

A great number of molecules present in our environment, i.e. water, foods, air can interact with the DNA macromolecule and give rise to adducts which are biologically non-compatible and will disturb the structure-activity relationship, alter the genetic information transmission and lead to mutagen, carcinogen or teratogen processes. Such interactions may affect the DNA template during replication, as well as mRNA, tRNA or one of their nucleotide precursors. Disturbances in the transmittance of genetic information, in the biosynthesis of amino acids – constituents of specific proteins – will lead to possible oncogen and teratogen effects. Damage to mitochondrial DNA (adducts and mutations), mitochondrial membranes, increased cell death (thanato-citosis) and disruption of ATP production can be produced, too. Biogenesis of DNA adducts occurs not only during the metabolization of nutrients but also in the case of the biotransformation of chemical xenobiotics (Poirier and Beland, 1992; Gârban, 2011). In fact, DNA adducts may be formed with various organic and inorganic compounds considered as xenobiotics of nutritional, pharmacological and toxicological interest. Further on structural data is presented on various xenobiotics and on structures of certain adducts formed after interactions.

3. Interactions with organic compounds

Interaction with aflatoxins

Data of FAO/WHO show that the number of known mycotoxins counts over 200 types, are the secondary metabolites of different varieties of moulds and highly toxic for both animals and humans (Bannett and Klick, 2003). These toxins are produced when moulds grow on agricultural products or after harvest or during transportation or storage.

Aflatoxins as secondary metabolites of fungi have been identified and isolated in the period 1961-1962. Aflatoxins – the most toxicogen mycotoxins, are produced by different species of *Aspergillus* as well as members of the species *Penicillium* and *Rhizopus*. *Aspergillus flavus* produces aflatoxins B_1 and B_2 while *Aspergillus parasiticus* produces aflatoxins B_1, B_2, G_1 and G_2. The interest for the knowledge of the chemical structure-biological activity as well as of the aspects related to the pathobiochemistry and

pathophysiology concerning mycotoxins generally, and aflatoxins especially, focused the theoretic and applicative scientific preoccupations on the environment, food quality and safety in relation with their effects on public health (Podar et al., 1982; Gârban and Daranyi, 2000). Aflatoxins are present in the food chain. They have been found in human cord blood and can enter into the developing fetus in humans and animals. Exposure to mycotoxins is mostly by ingestion (contaminated grain and cereals as well as contaminated animal products) but also occurs by the dermal and respiratory routes. Food contamination has been implicated in both animal and human aflatoxicosis. The toxic response varies in relation to species, sex, age, nutritional status, duration of intake and level of aflatoxin in the ration.

In the structure of aflatoxins (Fig. 2) one can remark monoheteroatomic heterocycles (furan, pyran), partially hydrogenated, where oxygen is the heteroatom. Structural formulas have been established by spectroscopy and crystallography. Aflatoxins belong to more structural types designated depending on: the blue fluorescence and noted AFB_1, AFB_2; the green fluorescence AFG_1, AFG_2 in ultraviolet light; according to the source of origin: milk AFM_1, AFM_2 and others: AFQ_1, AFP_1 .

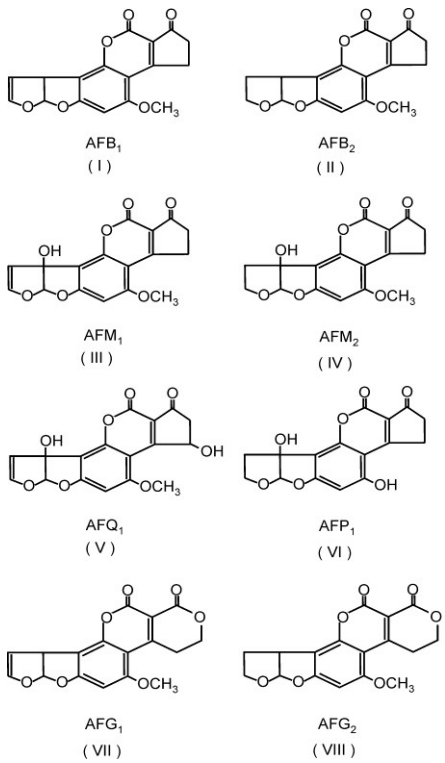


Fig. 2. Aflatoxins – structural formula

Beside the mentioned structures there have been isolated oxydrilated and methoxylated derivatives in various positions of the polycycles. Sometimes these have been considered new types of aflatoxins, but in fact they represent steps of the metabolization of initial compounds.

Predilectly AFB_1 and AFG_1 present high reactivity, which have an unsaturated side heterocycle (of furanic type). The biologic activity and toxicity decrease in the series: AFB_1 > AFG_1 > AFG_2 > AFB_2

Aflatoxin B_1 (AFB_1) is the major mycotoxin produced by most species under culture conditions. Because of this and its toxicity, B_1 is the most frequently studied. AFB_1 is a potent toxin ivolved in the ethiology of hepatocarcinoma. It is relatively non-reactive until it is ingested and converted by liver enzymes to the reactive intermediate AFB_1 8,9-oxide.This metabolite reacts with DNA and forms DNA-AFB_1 type adducts at the N_7 position of guanine.

The mechanism of action of aflatoxins consists in their interaction with the nucleic acids. During interaction, the extension and distorsion of the double helix takes place and, finally, the breaking of hydrogen bonds between the nucleobases A-T and G-C. Due to the high electron density of some atoms such as N_7 of guanine and O from C_4 of thymine, these ones present a high reactivity and accesibility for the interaction with an external chemical agent – see fig.3 .

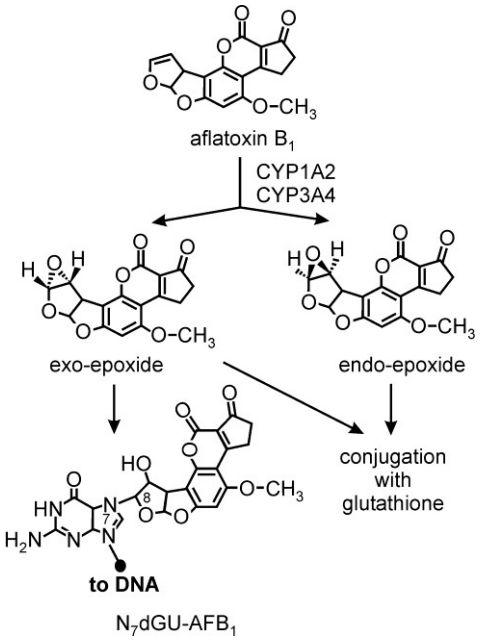


Fig. 3. Biotransformation of AFB_1 – genesis of glutathione-conjugates and DNA- AFB_1 adduct (type N_7dGu-AFB_1)

Human organisms metabolize AFB_1 to an 8,9-epoxide forming DNA and albumin adducts by the same activation pathways as susceptible animal species. Glutathione-S-transferase-mediated conjugation of glutathione 8,9-epoxide reduces DNA damage and this mechanism is important in reducing tumors.

These adducts formation explained by molecular pathology (pathobiochemistry), a complementary domain of cellular pathology, may be considered as precursory step of

morphofunctional changes observed in living organisms as a consequence of the action of mycotoxins.

Interaction with polycyclic hydrocarbons

Polycyclic aromatic hydrocarbons (PAH) have two important characteristics that influence their biological activity: a planar molecule and π delocalized electrons (Luch, 2005; Gârban, 2007). Deviations of the planar form can severely alter the chemical structure – biological activity relation. The structural formulas for polycene (I-XII) are presented in fig.4.

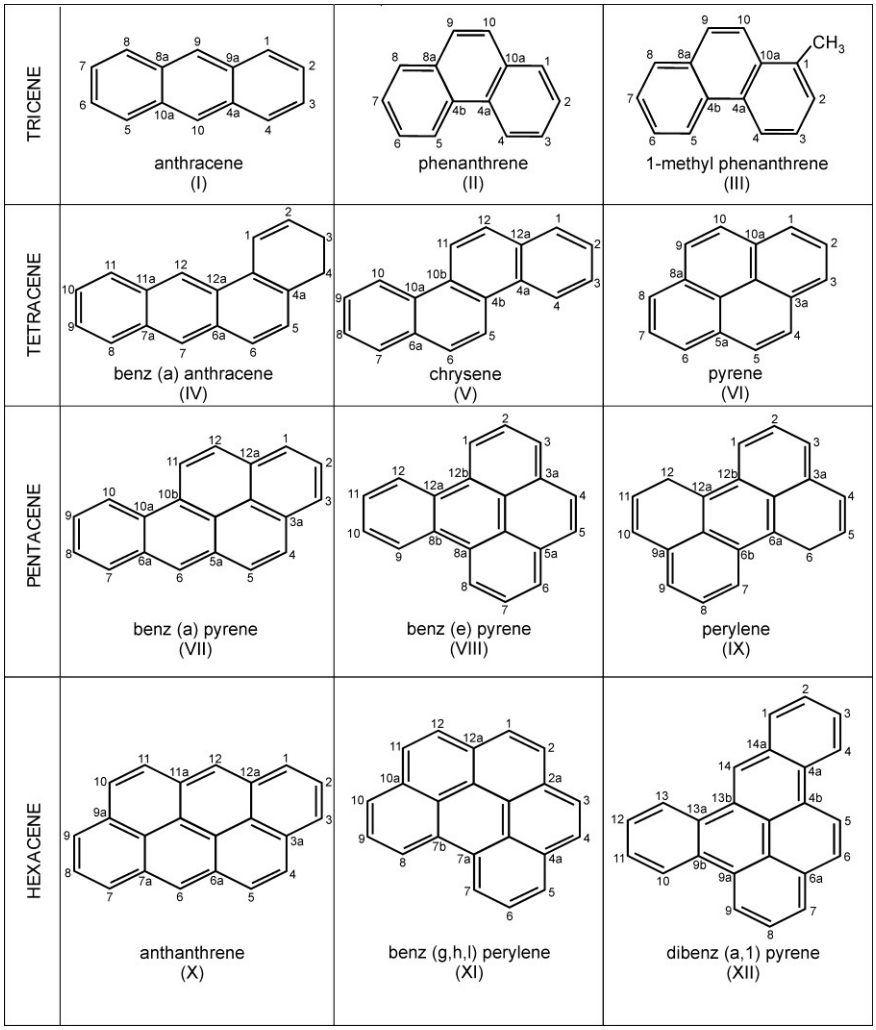


Fig.4. Polycyclic aromatic hydrocarbons – chemical structure

As a result of the DNA macromolecule interaction with PAH adducts of type DNA-HPA are formed (Arcos, 1996; Avacovici, 2005). Benz(a)anthracene (IV) – B(a)A and benz(a)pyrene (VII) – B(a)P are most often incriminated for their carcinogenic effects. In the case of B(a)P the binding to the DNA macromolecule usually takes place at N_2 of

deoxyriboguanine (N_2dGu) and at N_6 of deoxyriboadenine (N_6dAd) and in the case of MB(a)A the binding may appear at N_4 of deoxyribocytosine (N_4dCi) – see fig.5.

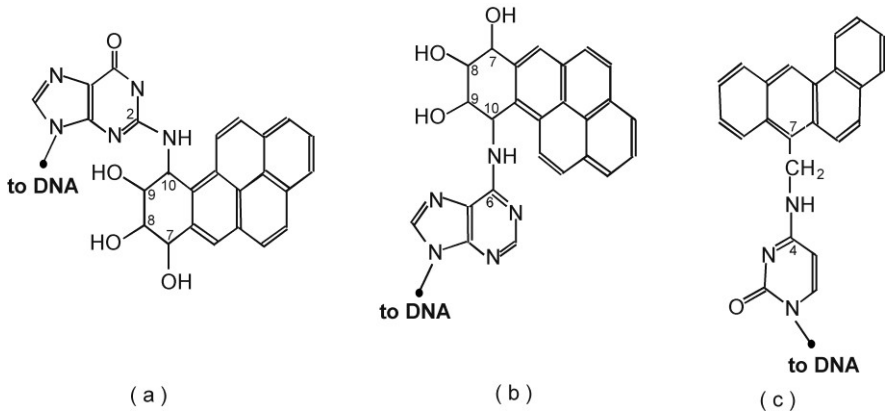


Fig.5. Structure of adducts DNA-PAH0 by bindings to nucleobases
(a)N_2dGu-B(a)P ;(b) N_2dAd-B(a)P; (c) N_2dCi-MB(a)A

Appearance of DNA-PAH adducts is followed by the destabilization of the DNA macromolecule which contributes to the perturbation of genetic information during the replication-transcription-translation processes, leading to the appearance of mutagenic and/or carcinogenic processes.

Interaction with aromatic amines

a) Interaction with aminobenzene

Aminobenzene ia an "azo dye" used in textile industry but in the past it has been used also in the food industry. It is known a monomethylated derivative (MAB) and a dimethylated derivative (DAB). Metabolization of MAB, for example, occurs at the level of hepatic ribosomes, giving rise to the biologically non-compatible adduct DNA-MAB. The binding is made at the C_8 of the deoxyguanosine of DNA, usually noted C_8dGu. Structure of the monoaminobenzene (MAB) and it adduct with DNA is given in fig.6.

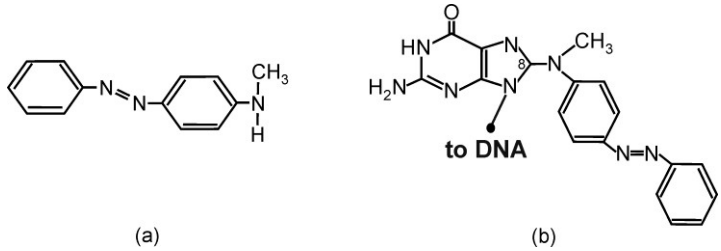


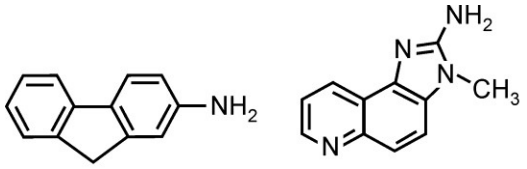
Fig.6. Structure of the monoaminobenzene – MAB (a) and of the adduct DNA-MAB (b)

b) Interaction with aminofluorene and with quinoleine

Aromatic amines are amines with an aromatic substituent – that is $–NH_2$, –NH – or nitrogen group(s) attached to an aromatic hydrocarbon, whose structure usually contains one or more benzene rings (Gârban, 2007). Human exposure to aromatic amines is made especially through industrial pollution and cigarette smoke. The biotransformation process

that activates the aromatic amines consist mainly in N-oxidation which generates N-hydroxy-arylamines that can interact directly with DNA, thus resulting DNA adducts.

Two of the most representative compounds from this class are 2-aminoflouren (2-AF) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) . Their chemical structures are given in fig.7.

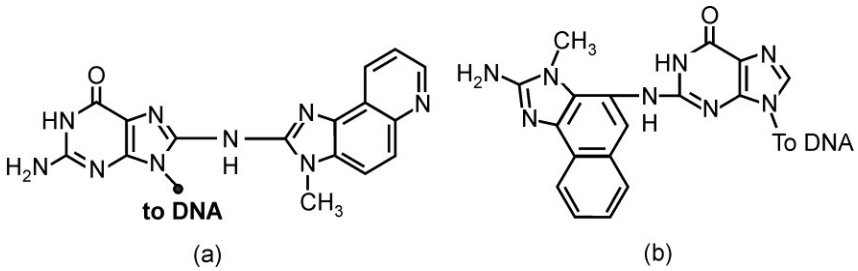


2-aminofluoren 2-amino-3-methylimidazo [4,5-f]quinoline

Fig.7. Chemical structure of 2-aminoflouren and 2-amino-3-methylimidazo[4,5-f]quinoline

By metabolic activation of aromatic amines electrophilic arylnitrenium ions are generated ultimately. These will attack DNA giving rise to specific adducts by binding to the DNA guanine and which will impede replication and induce mutations that underlie tumorigenesis.

One of the most recently discussed aromatic amines is 2-amino-3-methylimidazo-[4,5-f] quinoline (IQ), a carcinogenic compound found especially in cooked food. After a previous activation IQ interacts with DNA and generates especially N-(deoxyguanosine-8-yl)-2-amino-3-methylimidazo- [4,5-f] quinoline (C_8dGu-IQ) and 5-(deoxyguanosin-N_2-yl)-2-amino-3-methylimidazo- [4,5-f]quinoline (N_2dGu-IQ). Their chemical structure is given in figure 8.



(a) (b)

Fig. 8. Structure of some adducts between DNA nucleobases and IQ
a) C_8dGu-IQ; b) N_2dGu-IQ

The DNA-IQ adducts are incriminated especially for colon cancer and the structural formulas shown above were determined on lab rats after the administration of IQ.

4. Interactions with inorganic compounds

In this category the most discussed xenobiotics are the metallic compounds which may generate through biotransformation metallic ions that interact with the DNA macromolecule. Also in this category one can mention nitrates and nitrites which generate by biotransformation organic compounds like nitrosamines that interact with DNA.

Interaction with some metals

One of the most discussed and studied category of chemical xenobiotics are the potentially toxicogen metals, known for their carcinogenicity, and metals present in the environment as pollutants (especially of industrial origin) which can accidentally reach feedstuffs, food or tap water (Williams,1997; Merian et al., 2004; Haiduc, 2006). Among those metals the most common are: cadmium (Cd), lead (Pb), mercury (Hg) and tin (Sn).

Cadmium is used especially in the battery industry and in the pigments manufacturing, e.g. cadmium sulfide (CdS). Lead is found as an environmental contaminant, especially tetraethyl lead – $Pb(CH_2H_5)_4$ – from the emissions of auto vehicles. Mercury is an industrial pollutant – mostly discussed as a contaminant of sea food, especially as methyl mercury radicals (CH_3Hg^+) – e.g.: chloromethylmercury (CH_3HgCl).

After the interaction between DNA and metallic ions (M^{n+}), the resulted adducts severely alter the duplex stability. In some cases, when the metal ions are intercalated between nucleobases, the strand can be broken. The newly formed DNA-M^{n+} type adducts affect the structure-activity relationship of DNA macromolecule and the protein synthesis.

Divalent metal ions (M^{2+}) give rise to adducts with DNA by the binding to a nucleobase at N_7 position and O from C_6 of guanine and at N_7 and N from C_6 of adenine. An example of such adducts are those formed by the attachment of cadmium to guanine and adenine (fig. 9). Also, in case of Hg^{2+} and Cu^{2+} and the binding can appear at N_7 and O from C_6 of adenine or guanine.

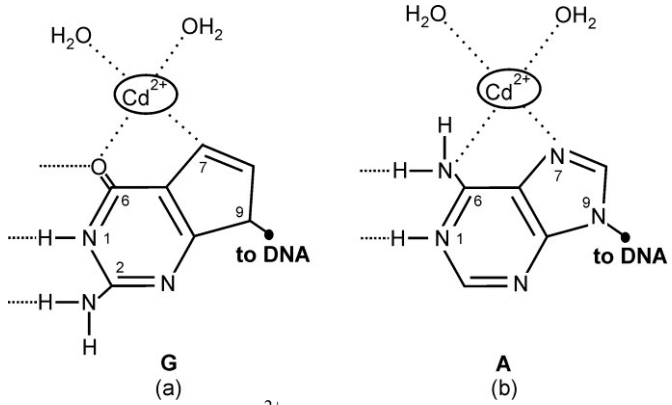


Fig.9. Binding of Cd^{2+} to purinic nucleobases of DNA:
a)Cd^{2+}-guanine; b) Cd^{2+}-adenine

The complexes formed by the interaction of DNA with divalent metal ions (M^{2+}) present various binding types: a) to the phosphodiesteric groups; b) between a phosphodiesteric group and nucleobases; c) between two complementary intrastrand nucleobases; d) between two vicinal nucleobases; e) at different positions of the same purine nucleobases. The first three binding modes are predilectly characteristic for biometallic ions.

Interaction with nitrates and nitrites

The nitrosamines may appear from the interaction with nitrates and nitrites. This interaction takes place usually during the digestion process. The formed nitrosamines have

non-cyclic (e.g.: N-nitrosodimethylamine, N-nitrosodiethyl-amine) or cyclic structure (e.g.: N- nitrosopyrolidine, N-nitrosopiperidine).

A special class of nitrosamines that is more and more studied these days due to their carcinogenicity and also due to their magnitude of impact on humans is the tobacco specific nitrosamines (Hecht, 1998; Mensinga et al., 2003). These nitrosamines are formed during the processes of curing and maturation of tobacco when nitrates are reduced to nitrites which act as nitration agents for the present secondary and tertiary amines. The most studied compound from this class is 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone also named N-nitroso ketone (NNK) which can form adducts with DNA. Among the resulted adducts the most common were those at the N_7 of deoxyguanine (N_7dG), N_2 of deoxycytosine (N_2dC) and N_2 of deoxythymine (N_2dT) – see fig. 10.

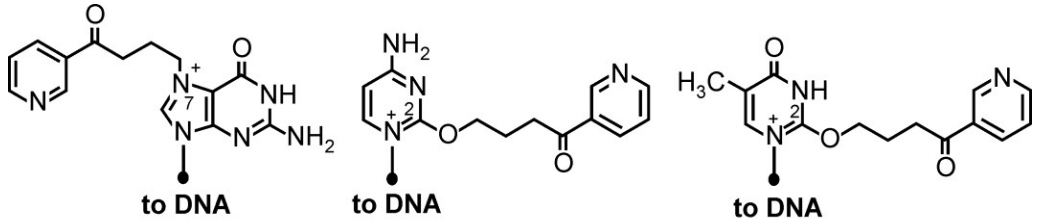


Fig.10. Chemical structure of some adducts between DNA and NNK:
a) N_7dG-NNK; b) N_2dC-NNK; c) N_2dT-NNK

These adducts were identified using mass spectrometric analysis and their presence is correlated with the initiation of carcinogenesis. Such adducts have been found in tissues and cells of rodents that have been exposed to NNK, and often in the blood and urine of smokers.

Compounds with adduct structure resulted from the interaction of DNA with various xenobiotics are non-biocompatible. In the organism such structures disturb the cell division and are followed by neoplastic processes. Aspects related to neoplastic processes will be discussed in another paper.

CONCLUDING REMARKS

Interactions of chemical xenobiotics with the DNA macromolecule are of importance both for fundamental studies – involving the molecular mechanisms and the applicative research - regarding the biochemical injury.

As a result of the biochemical injury is the formation of some biologically non-compatible “molecular complexes” known as DNA adducts. These compounds are at the origin of some processes which can affect the animal and human organisms. The effects are conditioned by the type of the xenobiotic, physiological status of the organism, access route a.o. In time, the pathobiochemical changes followed by pathophysiological modifications may lead to mutagen, oncogen and carcinogen processes.

In the domain of the carcinogen pathobiochemical processes the adduct formation of various chemical xenobiotics with DNA are of special interest. Investigations focused on the DNA adducts might help to improve diagnosis in the early stages of a carcinogenic process.

***Note**: The present paper was elaborated within the activity of the «Romanian Society of Comparative Oncology » Bucharest (Manolescu) and of the Working Group for Xenobiochemistry Timişoara (Gârban). Studies in the same domain were extended (after February 14, 2015) in the framework of the collaboration with the Faculty of Veterinary Medicine Timişoara.*

This work was communicated at the Jubilee Session of 70 yrs from the foundation of the University of Agricultural Sciences Timişoara (1945-2015).

References

1. Ames B.N., Shigenaga M.K., Gold L.S. – DNA lesions, inducible DNA repair and cell division: three key factors in mutagenesis and carcinogenesis, *Environ. Health Perspect*., 1993, 5, 35-44.
2. Arcos J.C., Argus M., Woo Y. (Eds.) – *Chemical induction of cancer: Modulation and Combination Effects*, Birkhauser, Boston, 1996.
3. Avacovici Adina, Gârban Gabriela, Ghibu G.-D., Gârban Z. – Adducts of deoxyribonucleic acid with polycyclic aromatic hydrocarbons and acridinic derivatives, *"Scientifical researches: Agroalimentary Processes and Technologies"*, Timişoara, 2005, XI (1), 129-138.
4. Bannett J.W., Klich M. – Mycotoxins, *Clinical Microbiology Reviews*, 2003, 16, 3, 497-516.
5. Brooks G.T., Roberts T.R. - *Pesticide Chemistry and Bioscience, The Food-Environment Challenge*, Publ. by The Royal Society of Chemistry, Cambridge, 1999.
6. Creppy E.E., Serge Moukha S., Bacha H., Carratu Maria Rosaria - How much should we involve genetic and environmental factors in the risk assessment of mycotoxins in humans? *Int. J. Environ. Res. Public Health,* 2005, 2(1), 186–193.
7. Gârban Z., Daranyi Gabriela - Biomedical implications of the adducts biogenesis resulted by the interaction of deoxyribonucleic acid with aflatoxins, *Central European Journal of Occupational and Environmental Medicine,* 2000, 6 (2-3), 161-167.
8. Gârban Z. - *Tratat comprehensiv de biochimie. Vol.IV. Xenobiochimie*, ediţia 2-a, Editura Didactică şi Pedagogică R.A., Bucureşti, 2007.
9. Gârban Z. - *Xenobiochimia în ştiinţele agricole şi ştiinţa alimentului*, Editura Solness, Timişoara, 2011.
10. Haiduc I. – Metals in our food, pp.15-20, in "*Metal Elements in Environment, Medicine and Biology",* Tome VII (Gârban Z., Drăgan P., Eds.), Publishing House Eurobit Timişoara, 2006.
11. Hecht S.S. – Biochemistry, biology and carcinogenicity of tobacco-specific N-nitrosamines, *Chem. Res. Toxicol*., 1998, 11, 559-603.
12. Knudsen I. – Animal Studies in the Evaluation of Carcinogens - Anticarcinogens in Food, *Biol. Zent. bl*., 1989, 108, 423-450.
13. Kohlmeier M. – *Nutrient metabolism, Chap. 5, Xenobiotics* (pp.85-110), Academic Press, Amstedam-Boston-Heidelberg-London-New York, 2003.
14. Luch A. - *The Carcinogenic Effects of Polycyclic Aromatic Hydrocarbons*, Imperial College Press, London, U.K., 2005.
15. Manolescu N. (Ed.) - *Introducere în oncologia comparată*, Editura Universitară "Carol Davila" Bucureşti, 2003.
16. Mensinga T.T., Speijers G.J.A., Meulenbelt J. – Health implications of exposure to environemental nitrogenous compounds. Review article, *Toxicological Reviews*, 2003, 22(1), 41-51.
17. Merian E., Anke M., Stoeppler M. (Eds.) – Elements and their compounds in the environment. Vol.I, General aspects (2nd edition,Viley-VCH Verlag, Weinheim, 2004.
18. Nassar A.F. (Ed.) - *Biotransformation and Metabolite Elucidation of Xenobiotics: Characterization and Identification*, J.Wiley and Sons Inc., Chichester, UK, 2010.
19. Phillips D.H. – DNA adducts as markers of exposure and risk, *Mutat. Res.,* 2005, 577(1-2), 284-292.
20. Podar C., Gârban Z., Silvaş E., Roman M., Ciorlăuş A.T., Ştef L. - Poluarea micotică şi implicaţiile acesteia în patologia tineretului taurin, *Rev. Creşt. Anim.,* Bucureşti, 1982, 2, 2, 46-50.

21. Poirier M.C., Beland F.A. – DNA adducts measurements and tumor incidence during chronic carcinogen exposure in animal models: implications for DNA adducts-based human cancer risk assessment, *Chem.Res.Toxicol*., 1992, 5, 749-755.
22. Wassom J.S. - *Mutagenesis, carcinogenesis and teratogenesis information system*, Information Center Complex, Oak Ridge, 1980.
23. Williams R.J.P. - Bioinorganic Chemistry and Cancer, pp.19-83, in "*Carcinogenicity of inorganic substances: Risks from Occupational Exposure"* (Chief Duffus J.H., Ed.), Publ. by The Royal Society of Chemistry, Cambridge, 1997.