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Oxidative DNA damage

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Several characteristics of the basic types of oxidative DNA damage are analysed in the present work. They are as follows: base and sugar modifications lesions, single-strand and double-strand breaks, apurinic/apyrimidinic sites and DNA-proteins cross-links. The chemical structure of the most investigated types of oxidative DNA damage is shown. The most common genotoxic agents (reactive oxygen species, free radicals, alkylating agents) are also discussed. The methods of identification and measurement of oxidative DNA damage are considered.

Key words: oxidative stress, DNA damage, genotoxic agents

Introduction. A vast number of different types of DNA damage take place in the cell at normal conditions. However, a strict boundary line has to be drawn between DNA damages at normal conditions and artificial damages occurring in the vial.

DNA damages may occur due to various chemical and physical agents of exogenic or endogenic origin. Exogenic agents, capable of damaging DNA structure, include the following: ionizing radiation, ultra-violet radiation, some medications, staining agents, *etc*. Endogenic DNA damaging agents, *i.e.* active forms of oxygen and nitrogen, free radicals, methylating agents, even at normal metabolic conditions form a significant number of DNA damaging factors. All these compositions cause the damage of DNA as a result of reactions of alkylation, hydrolysis, and oxidation, and require reparation. At the same time single- and double-strand breaks, base drop-out sites (AP-sites), base and sugar modifications lesions, intervalent interaction with proteins (cross-links) are formed. Some of the mentioned damages may be repaired and some of them may not. It is evident that such structural changes will influence the functions of DNA. Meanwhile, there is always a certain level of modified DNA in the cell [1–5].

Current review presents the information on oxidative DNA damages only, occurring both during normal metabolism and oxidative stress conditions. The range of these damages is rather wide (it includes nitrogen bases modifications, changes in deoxyribose structure, chain breaks), therefore, talking about oxidative DNA damages, they have to be considered as a complex of different damages, caused by the group of particular agents [1–5].

Types of oxidative DNA damages and the groups of agents which cause them. DNA damages occur as a result of a series of chemical reactions, *i.e.* redoxreaction, alkylation, hydrolysis, *etc*. The cells always demonstrate a balanced level of DNA damage. The level of DNA damages, which exceeds the norm 10-100 times, and not the presence of a certain type of damage, is considered to be the pathological condition index [1–6].

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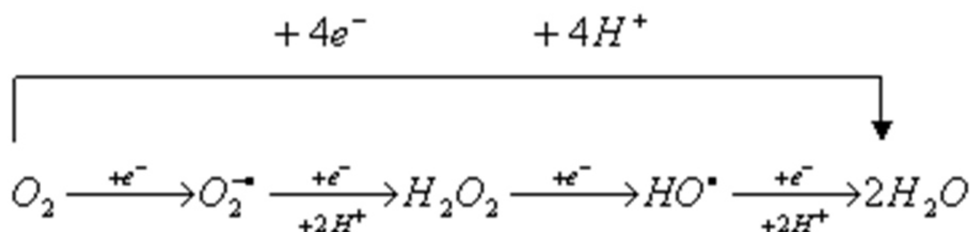

Fig.1 AFO formation during four-electron oxygen restoration in the cell

Oxidative DNA damages are numerous in the course of oxidative stress under the influence of free radicals and other active forms of oxygen [1–7]. Free radicals, which contain unpaired electrons, are dangerous and surprisingly reaction-capable compounds. They are formed throughout the time of metabolic transformations in mitochondria, endoplasmic reticulum, *etc*. Regardless of the short half lifetime (1 nsec for OH•-radical), free radicals are of significant influence on macromolecules of the organism [2, 8]. Simple calculations reveal that at the diffusion level of 500 m/sec and the size of the molecule with an exited atom or an exited chemical group of 0.5 nm, in 10^{-9} sec a free radical can cover the distance of $0.5{\cdot}10^{12}$ nm, which is ~1000 of its diameters [9]. Free radicals also include some active forms of oxygen (AFO), nitrogen (AFN), lipid radicals (peroxide – ROO•, alkoxyl RO•, *etc*), as well as some semiquinones (•QH). Interacting with a DNA molecule, free radicals may become the reason of chain breaks and modification of bases and deoxyribose [2, 5].

AFOs represent rather a wider collection, compared to free radicals, as this group includes not only free radicals of oxygen (superoxide-anion O_2^- and hydroxyl-radical OH•) but also hydrogen peroxide, singlet oxygen (with electron of singlet condition, $^1O_2$, compared to normal, the triplet one), ozone, and some “non-classical” AFOs [1, 5, 7]. These compounds are capable of causing the largest group of damages – the changes in structure of nitrogen bases, deoxyribose, single- and double-strand breaks, intermolecular cross-links. All these damages are considered as the oxidative stress indices, which have the negative influence on the cell, as well as on the whole organism [1–7].

The biggest contribution into the endogenic formation of active forms of oxygen is made by mitochondrial respiratory chain (that is why mitochondrial DNA is often selected to be the object of oxidative damage investigations), the system of cytochrome P-450 in the endoplasmic reticulum, and в-oxidation of fatty acids. These compounds are formed in the course of other metabolic reactions as well, and some cells of immune system (macrophages, neutrophils, and eosinophils) neutralize allogenic agents by AFOs. Exogenic stimulators of AFO formation may also include UV rays, ionizing radiation, and some chemical compounds [1, 5, 6, 8]. On entering the organism, the oxygen molecule has to transform into water, by means of connection of 4 electrons and 4 protons, however, even at normal conditions, 5% of oxygen connects not 4, but 1, 2, or 3 electrons, which results in the formation of AFOs, *i.e.* free super oxide-anion O_2^-, hydrogen peroxide (H_2O_2) and the most effective hydroxyl-radical (OH·) (Fig.1) [8, 10].

Hydrogen peroxide is of low reactional capability, but in the presence of metals of altered valence (iron, copper) it takes part in, so called, Fenton’s reaction [11]:

$$Fe^{2+} + H_2O_2 \quad Fe^{3+} + \ OH + OH^-$$

One more reaction is Harber-Weiss’ reaction, accompanied by the formation of a significant amount of OH-radicals:

$$O_2^- + H_2O_2 \quad O_2 + \ OH + OH^-$$

At normal conditions, peroxides are neutralized by enzymatic systems (superoxide dismutase, glutathione-peroxidase, catalase, peroxidase) and oxidative stress protection is provided by the complex of natural antioxidants, such as tocopherol, ferritin, carotenoids, ascorbic and uric acids, *etc* [13].

OH-radicals interact with deoxyribose with a subsequent formation of various derivatives (erythrose, 2-deoxy-tetrodialdose (Fig.2)) and chain breaks, while

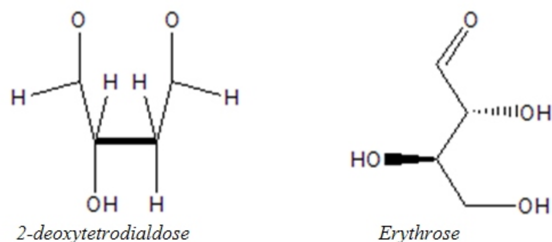

Fig.2 Deoxyribose derivatives, formed as a result of hydroxyl radical attack

their interaction with all types of nitrogenous bases results in the formation of hundreds of intermediate products [2].

Due to instability of the majority of formed intermediate products the biochemical specificities of only some of them were defined clearly. The main forms of oxidative damages of nitrogenous bases are shown in Fig. 3 [1, 5].

The vast majority of damages, caused by OH-radicals, were studied *in vitro* and *in vivo* using -irradiation [1, 3]. Hydroxyl-radical is capable of ripping of the hydrogen atom from thymine methyl group and from each one of C-H bonds of 2'-deoxyribose [1]. Coupling of double bounds in the bases is characterized by specific velocity constant of $3\text{-}10 \cdot 10^9$ $M^{-1} \cdot sec^{-1}$ and hydrogen atom ripping velocity was shown to be $2 \cdot 10^9$ $M^{-1} \cdot sec^{-1}$. C4-C5 is OH-radical sensitive in pyrimidines whereas C4, C5, C8 – in purines. The attack of hydroxyl-radical results in different modifications of pyrimidine bases, *i.e.* formation of 5-OHdU (Fig.3, *b*), 5-OHdC (Fig.3, *d*), uracyl glycol (Fig. 3, *e*), thymine glycol (Fig. 3, *f*) and some other compounds [1–7].

The interaction of OH-radical with purines also leads to some changes – the formation of formamidopyrimidine (Fapy), 8-oxoguanine (8-OHdG) (Fig. 3, *a*) (basic oxidative stress marker *in vitro* and *in vivo*), 8-hydroxideoxyadenosine (8-OHdA) (Fig. 3, *c*), *etc.* Fapy is an open imidazole circle guanine 2,6-diamine-4-hydroxy-5N-methyl-formamidopyrimi dine (Fapy-G) (Fig. 3, *g*) and 4,6-diamino-5-formamidopyrimidine (Fapy-A) (Fig. 3, *h*) [1]. The formation of 8-OHdG or almost equivalent 8-oxo-7,8-dihydroguanine (8-oxo-G) is the most common result of oxidative damages. All these compounds turn easily into one another, thus, they are usually called 8-oxo-G. The quantitative analysis of 8-oxo-G is most commonly carried out in order to determine the level of oxidative stress. It comes from high contents of these compounds in the cells as well as the presence of a large amount of relatively objective research methods [1–7]. The whole series of commercial kits for quantitative analysis have been developed nowadays [1, 4, 14, 15]. Glycols and hydrates of cytosine lead to transversion as a result of aminogroup ripping more often, compared to normal cytosine [1, 16]. Additional compounds of nitrogenous bases with OH-radical is the group of compounds having oxidising/reductive ambivalence and capable of being a part of complex reactions [1].

Some modified bases become free radicals and result in chain reactions. For instance, cytosine radicals C5-OH-6-peroxile and C6-OH-5 are involved in the formation of 4-amino-5-hydroxy-2,6(1H,5H)-pyrimidine dion and 4-amino-6-hydroxy-2,5(1H,6H)-pyrimidine dion [1, 3, 5].

DNA interacting with ozone, singlet oxygen and other AFK, also results in the formation of a series of nitrogen bases derivatives [3, 4].

Often nitrogen oxide (NO) is formed in the cell out of arginine accompanied by NO-synthetase. Its [arginine] interaction with DNA results in deaminization of bases and occurrence of transitions [6].

Hypochlorite (ClO^-) induces the damage spectrum, similar to the effect of singlet oxygen [3, 4].

Ultra-violet light induces the formation of so called pyrimidine dimers of two types (Fig. 4) [17, 18].

Visible light brings up the reactions, identical to those caused by singlet oxygen and fluorine reactions of type I (connected with the effect of photo sensitizers, *e.g.* hematoporphyrin, riboflavin, methylene blue, *etc*) [3, 4]. However, similar reactions are different from those caused by OH-radical, which causes almost equal amounts of breaks, AP-sites and different modifications of bases. Meanwhile, singlet oxygen and the majority of photosensitizers lead to damages, basically, sensitive to the effect of enzyme of excision repair of formamidopyrimidine-DNA-glycosylase, 8-oxo-G and formamidopyridines [3, 4, 18, 19].

A significant number of oxygen active forms appears during the inflammatory processes, which is the

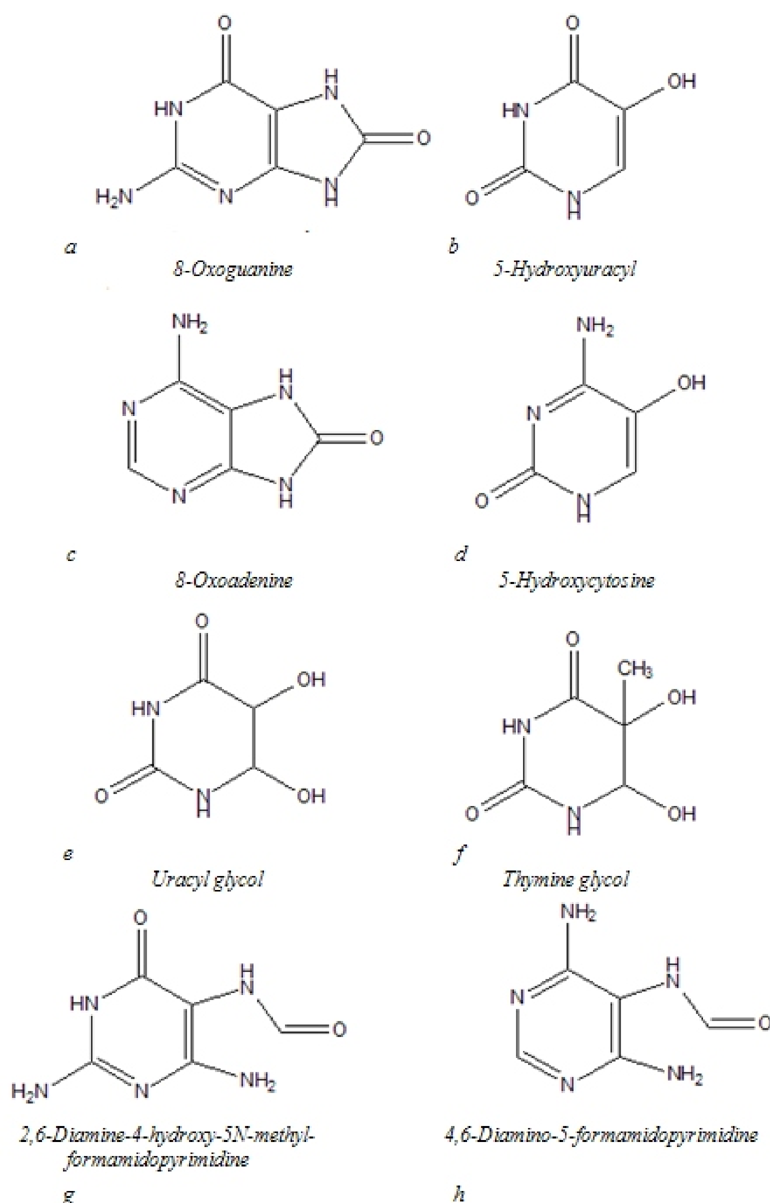

Fig.3 The most common modifications of nitrogenous bases as a result of AFO effect

result of the activity of cells of immune system. NADN-oxidase enzymes and myeloperoxidase, which form OH-radicals and hypochlorite, which, in a turn, are involved in elimination of allogenic agents, take an active part in the processes of activation of macrophages. Therefore, the oxidative stress is considered to be one of the indicators of chronic inflammations [1, 6, 10].

The intervalent interactions of nucleic acids and proteins represent one more type of free radicals-induced damages (especially, by hydroxyl radical and malondialdehyde) [1, 2]. The most vivid examples of this group are thymine-tyrosine and thymine-lysine interactions. Thymine is also capable of interacting with glycerol, alanine, valine, leucine, isoleucine, and threonine. Sometimes cytosine interacts with tyrosine [2].

DNA alkylation is the process of spontaneous (without any interference from the enzymatic systems of organism) binding of alkyl group in certain position of the nitrogenous base [20]. Alkyl group may be

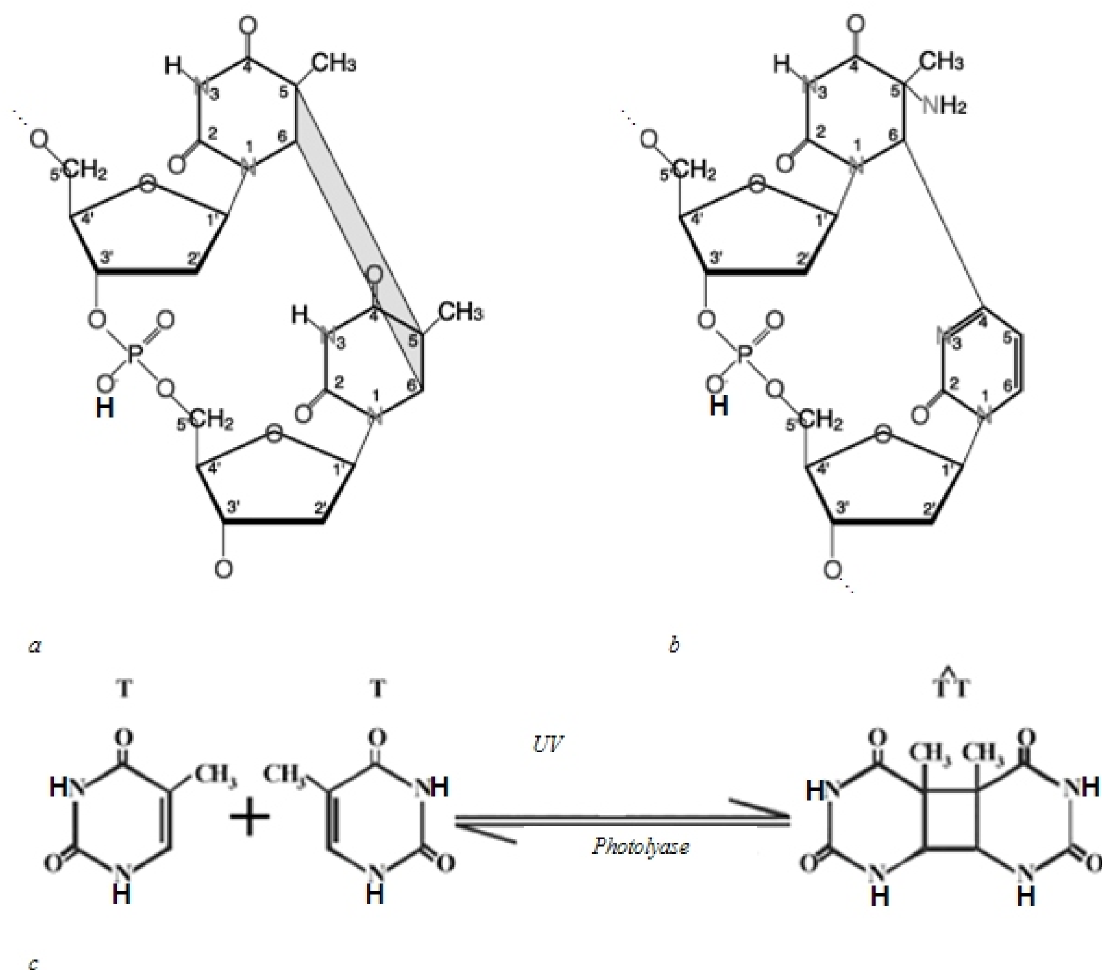

Fig.4 UV-formed products: *a* – cyclobutane dimers, *b* – 6-4-dimers, *c* – thymine-thymine dimers

shifted in the form of carbanion, carbcation or free radical. Alkylating agents comprise rather a large group of compounds capable of damaging the structure of macromolecules. They can also bind alkylating side groups (methyl, ethyl, propyl, butyl groups, *etc*). Alkylating factors are classified according to the type of the transferred group, and also according to the number of such groups (mono-, bi-functional, *etc* agents). The most common to be bound are the methyl groups [20-23]. DNA alkylation is not the subject of current research, however, some of its basic features are going to be presented, as oxidative stress results in increase in DNA damages by other agents as well [5].

Alkylating agents can be of exogenic and endogenic origin. Exogenic ones include epoxides, в-lactones, diazocompounds (diazomethane ($CH_2N{=}N$), nitrocompounds (N-methyl-N-nitro-N-nitrosoguanidine $CH_3N(NO)C(=NH)NHNO_2$), *etc*). The latter is considered to be one of the most dangerous exogenic alkylating agents, at the same time, S-adenosylmethionine is one of the most dangerous ones among endogenic alkylating agents. Some of the agents have been used as anti-tumour drugs [20].

O^6-methylguanine, O^4-alkylthymine, 3-methyladenin, 7-methylguanine, and 7-ethylguanine, capable of binding thymine (Fig. 5), are considered as the most common products of alkylation of nitrogenous bases [20].

N-1, N-2, N-3, N-7, O-6 guanine, N-1, N-3, N-6, N-7 adenine, N-3, N-4 O-2 cytosine, and N-3, O-2, O-4

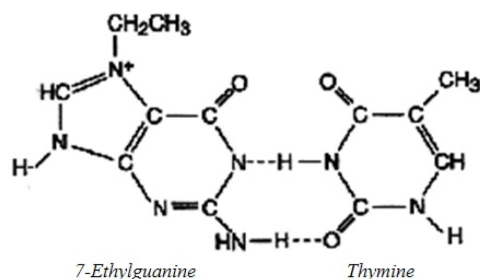

Fig.5 The formation of hydrogen bonds between electron-neutral 7-ethylguanine and thymine

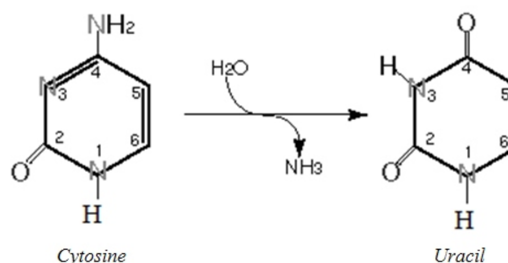

Fig.7 Deaminization of cytosine with uracyl formation

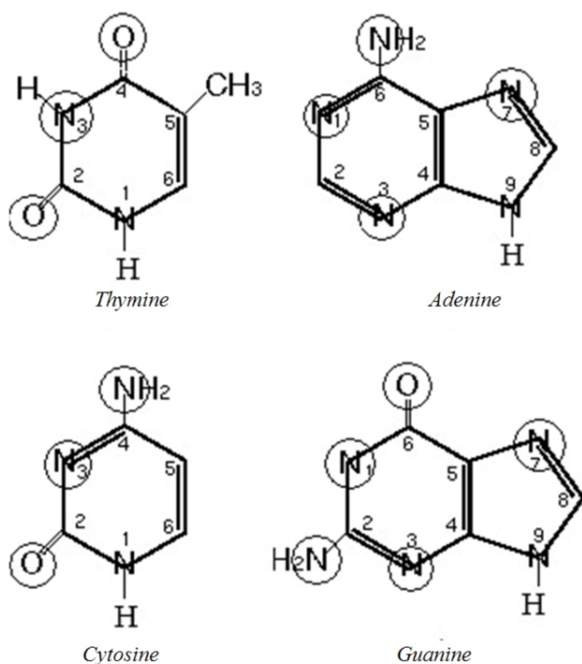

Fig.6 Sites of possible alkylation of nitrogenous bases (circled)

thymine are the sites, alkylation of which may result in mutations (Fig. 6) [21-23]. The specificities of DNA alkylation are described in some reviews [24, 25].

The hydrolysis-caused damages result in several types of damages: deaminization, depurination, and depyrimidination, *i.e.* the formation of lost bases sites (AP-sites) [1, 20].

Uracil is formed as a result of loss of the amino group out of cytosine (Fig.7), xanthine out of guanine, and hypoxanthine out of adenine [16]. Deaminization of cytosine and its derivatives is considered to be the most dangerous one. Thymine is formed as a result of deaminization of 5-methylcytosine (Fig.8) [20].

The appearance of AP-sites is rather a common group of damages. Purine bases are lost easier than pyrimidine ones. These kinds of process often take place in brain, heart, liver, intestinal canal, and not so often – in kidneys and lungs. Also AP-sites appear as a result of the effect of reparation enzymes. For instance, glykolases cut out the modified base after the appearance of AP-site. The reason of their formation is the hydrolysis of N-glycosyl bonds as well as the attack of free radicals in positions 1', 2' or 4' of deoxyribose (Fig.9) [5, 26, 27].

Methods of quantitative and qualitative analyses of DNA damages. The methods of investigation of oxidative, as well as other DNA damages may be divided into two groups. The first group includes the methods, application of which requires hydrolysis of molecules, the second one includes the methods used to study the whole molecule [5, 7, 13, 17].

In order to perform fluorescent, chromatographic and some radiological investigations, DNA has to be prior chemically or enzymatically hydrolysed. The largest subgroup is the group of chromatographic methods, the most effective one among which is the method of high resolution liquid chromatography, combined with electrochemical detection (HPLC-ECD), as well as the method of gas chromatography with mass-spectrometry (GC/MS) [3, 5, 7, 13, 17, 28].

The application of the second group of methods requires some special enzymes [3, 5]. After the enzymatic treatment, the material is analysed using single cell gel-electrophoresis (SCGE), method of alkaline elution [29] *etc*.

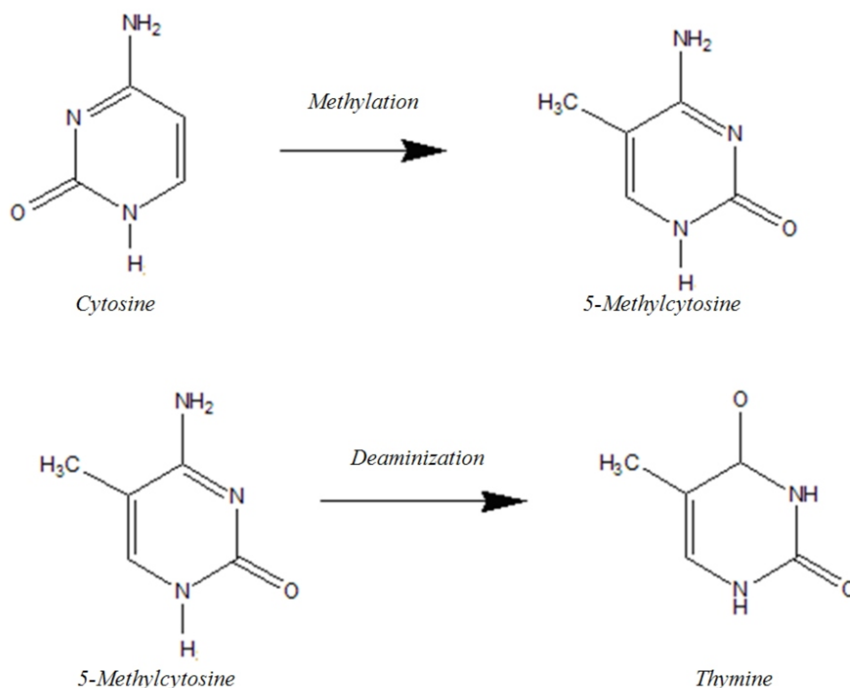

Fig.8 reactions of methylation and further deaminization of cytosine

The application of such enzymes as formamidopyrimidine-N-glycosylase (*fpg*), as well as its eukaryotic homologues (particularly, hOGG1), and endonuclease III is rather popular [30-33]. There is a whole series of commercial kits for determining the DNA damages using the enzymes. Bacterial glycolases are the most commonly used, however, some manufacturers supply their kits with human and yeast homologues of these enzymes [17, 31]. *fpg* is a very convenient application-wise enzyme, which consists of 269 amino acid residues and its molecular weight is 30.2 kDa. Protein coding gene consists of 807 pairs of nucleotides. *fgp* recognises 8-oxo-G, formamidopyrimidines (purines with open imidazole circle and) is capable of cutting out AP-sites, *i.e.* is of lyase activity. *fgp* is maximally active at pH values in the range of 6.5 to 8.5 and *fpg* does not require two-valence cations [30, 33].

The method of comet targeting consists in the assessment of the degree of DNA damage, based on the correlation of the length of the “tail”, which is formed during the movement of the damaged nucleic acid regions during electrophoresis, and the diameter of the nucleus, where undamaged DNA is concentrated [13]. Fluorescent dyes, *i.e.* acridine orange, ethidium bromide, propidium bromide, and the most recent one SYBR®Green, are used for visualisation [34].

Complete DNA is analysed by immunological methods as well. These methods include ELISA tests (*e.g.* ARP-test), radio-immune analysis, and Immune slot blot method, all of them are rather convenient, although, not sensitive enough for qualitative analysis [16, 17, 35].

The methods used to study the damages of genetic material, according to the type of interference, are classified into invasive and non-invasive (the latter include the determination of the amounts of damaged DNAs in urine) [17, 26, 36].

The selection of methods which are the most rational to be used for studying DNA damages is a very difficult task. The search for the most adequate method has been in process for several decades, however, the problem remains unsolved. The difference between the results, obtained using several different methods, is amazing [17]. Thus, the data obtained using GC/MS, reveal the level of 8-OHdG in the cells to be at the hundreds of residues per 10^6 of normal guanines. HPLC-ECD allowed obtaining the result being at ~5–50 residues per 10^6 of guanines. The enzymatic research using *fpg* and subsequent application of methods

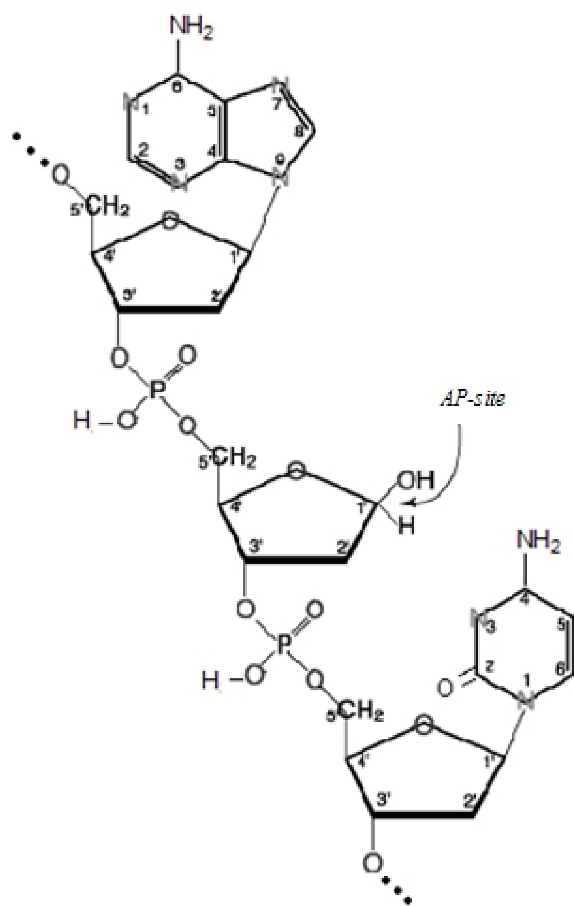

Fig.9 Scheme of possible AP-site

of comet targeting or alkylating untwisting revealed 0.5 of 8-OHdG residue per 10^6 of guanines [3, 13, 17, 28, 37, 38]. Presented disarrangements of the results are explained by the appearance of artefacts in the course of investigation, related to the preparation of the material, DNA isolation, *etc*. Besides, the state of the investigated molecule depends on the presence of certain enzymes, endonucleases in particular [3, 28, 37, 38].

The method of quantitative polymerase chain reaction (Q-PCR) has become popular in the recent years. This method is based on the capability of some forms of DNA damages to block replication, decreasing the amplification efficiency [7, 37].

Some qualitative data are presented in literature sources. The number of events, capable of provoking a destructive influence on the genetic material of cells varies, according to different data, from 74,000 to 500,000 per twenty-four hours [28]. It is known that the formation of 8-OHdG, or almost identical to it 8-oxo-G, is the most common result of oxidative damage (from 7,500 to 200,000 modifications of the cell per day) [16, 28]. The frequency of 7-methylguanine formation in the cell is app. 4,000 events per day. 3-methyladenine is considered to be one of the most common modified bases with high formation frequency (several hundreds events per day) [39]. The appearance of AP-sites is one of the most wide-spread forms of damage – around 200,000 bases are lost a day. Some data present the level of spontaneous depurination at normal conditions to be at 10,000 bases per day [27].

A series of problems does arise when the number of certain damages (8-oxo-G, for example) has been estimated. A number of works with artificially synthesised oligonucleotides with a known amount of 8-oxo-G were carried out [17]. The results obtained demonstrate that HPLC method underestimates the level of damages detected significantly. The investigations which involved phenol for protein purification, revealed the opposite results, *i.e.* significant overestimation, as it is commonly-known, phenol is capable of damaging nucleic acids. The application of sodium iodide revealed the lack of 8-oxo-G, which may be explained by the capability of sodium to repair the damages [3, 13, 17, 40].

Some scientists consider the determination of the DNA damages in the intact cells to be the best way to avoid the aforementioned problems. Immunological methods are suitable for these purposes, although, the use of antibodies is rather efficient for visualisation of damages, yet it remains semi-quantitative one. The method of comet targeting is a convenient method, but the application of this method underestimates the amounts of 8-oxo-G as well. Possibly it is due to the fact that the enzymes do not reach the chromatin "depths" and two closely located damages may be cut out as one. One more popular method is the method of GC/MS, but it has earlier been mentioned to overestimate the quantitative indices [7, 17, 28, 40, 41]. Therefore, nowadays neither one of known methods provides correct data on the numbers of DNA damages due to significant disarrangements of the results, which leads to the use of comparative analysis, regardless of the indication of exact numerical values [7, 13, 17, 28].

It is worthy to be noted that today special attention is paid to the studying of the damages of mitochondrial

DNA. The degree of its (mitochondrial DNA) oxidative damage can be interpreted as the index of organism age. However, on the other hand, the isolation of mitochondrial DNA as such is accompanied by significant oxidative damage, which overestimates the indicated level of mitochondrial damages essentially [37, 38, 40, 42].

Therefore, there are no clear data on the number of 8-oxo-G as well as other modifications in normal young, ageing, or sick cell at the moment. All the results may be considered to be of relative correctness due to the absence of one and common recalculation coefficient for the number of damages. The difficulty of obtaining quantitative data is accompanied by the fact that exact numbers of damages in the cell under the influence of a certain agent may be observed at the moment of the effect of this agent only, as the reparation systems are working constantly. Thus, the methods of studying DNA are being improved and modified with the purpose of achieving higher levels of their sensitivity, as well as of maximal decreasing the level of damages in the course of preliminary treatment of the material and elimination of artefacts [1, 3, 5, 7, 17, 28]. Often there is a question, arising in the course of determining certain damages, oxidative ones, for instance, occurring in the cell – Is DNA damage a reason or a consequence?

Maintaining the integrity of genome is the moment of special importance for proper functioning of organisms, which is complicated by the presence of the whole series of various factors, capable of loosening the genome consistency.

A number of works are dedicated to the biological consequences and the importance of oxidative damages of DNA, however, the present review shall present only some of general notions.

The problem of oxidative stress is considered to be one of the most current biological problems in the course of the recent decades. The damages of the genetic material, occurring as a result of the effect of active oxygen forms on DNA, are considered to be one of the constituent parts of this notion [1–7, 17].

The question of the role of oxidative damage of DNA in the processes of mutagenesis, carcinogenesis, and ageing, attract the most of attention nowadays [1–7, 12, 13]. A series of publications have been dedicated to the investigation of free-radical theory of age-related changes in the organism. Unfortunately, the application of various methods of quantitative analysis of oxidative stress markers (*e.g.* 8-OHdG) provides different results [1, 7, 17, 28]. However, evident is the fact that some modifications result in mutations, stimulate carcinogenesis, activating proto-oncogenes and inhibiting cancer-suppressors, influence the regulation of cell cycle, the course of transcription and replication processes, and participate in the development of ageing processes [1, 3, 7, 43]. It is also known that some DNA damages occur in the cases of cardiovascular diseases, nervous system diseases *etc*. One of the hypotheses of ageing is the hypothesis of inhibited reparation system with the course of time (ageing), which results in accumulation of errors in DNA [1–8, 32].

As of today, there is not a single doubt that chemical reorganisation of DNA may result in significant changes, *i.e.* transitions, transversions, and deletions [1, 3, 4]. It has been discovered that the highest mutagenic capacity is specific to O^6-methylguanine and O^4-alkylthymine [20–22]. 8-oxo-G is also a mutagen and, as a part of nucleoside triphosphate, it is mounted into DNA on the opposite side to adenine on the DNA template, resulting in G:C T:A transversions [1–7]. However, 8-oxo-G does not block reparation and transcription and has no influence on the cell cycle [3], opposite to thymine glycol, a rather common damage, which hinders the replication and is considered to be potentially lethal for the cell [1]. Formamidopyrimidines have got the capacity to block polymerases as well [1, 4]. Deaminization of 5-methylcytosine is of mutagenic nature, which results in the formation of T:G pair [1, 20]. Besides mutagenic capacity, damages of DNA activate the process of malignization of cells [1, 6, 12, 44]. Carcinogenesis may take place in two possible scenarios – some DNA damages are capable of activating proto-oncogenes, *p21*, *c-myc*, *c-Ha-ras*, in particular, or inhibit cancer-suppressor genes, e.g. *p53*, *Rb* [43, 45, 46]. Valid data testifying to the presence of 8-OHdG surplus *in vivo* in *ras*-oncogene and cancer-suppressor gene p53 in cases of lung, liver, and intestine cancer have been obtained [47–51]. Breast cancer has been proven to be connected with the accumulation of DNA damages due to oxidation and alkylation [52]. The link between the amounts of oxidative damages of DNA due to ageing and prostate cancer has been revealed [53].

There are two known types of effect of modified DNA on cell cycle – the accelerating one, causing malignization, and the decelerating one, resulting in apoptosis [1, 42, 54].

It has to be emphasized specifically that the works of the last three years reveal a growing number of oxidative damages of DNA in the course of inflammatory processes. As a result, a clear cause-and-effect connection between the condition of the cell and the condition of its genetic material is evident [1, 5, 6, 28].

The processes of ageing are generally related to both programmed events and accumulation of errors. Free-radical theory of ageing, or oxidative stress theory, is considered to be the most popular one as it includes the provisions attempting to explain programmability and accumulation of errors [42]. According to the given theory in the course of the life of organism (even at normal metabolism) a great number of free radicals are formed, which include active forms of oxygen, causing the damage of biological macromolecules with subsequent disordering of regulatory processes [1, 15, 40, 42, 44]. The hypothesis of possible role of free radicals in the processes of ageing has been proposed by Garman in the 1950s. In 1990 the possibility of effect of DNA oxidation on the process of ageing has been defined clearly [36, 40]. The results obtained demonstrated a 2-3 times increase in 8-oxo-G amount in experimental rats. However, these results turned out to be not persuasive enough, leaving the room for doubts due to the fact that the investigation was carried out on DNA, isolated by using phenol, and as a result the occurrence of artefacts was highly possible [1, 3, 7]. Some scientists consider the investigation of DNA, isolated using sodium iodide to be more convincing. This method allows obtaining more accurate results without any additional damages [14, 17]. According to these data, the level of oxidative damages of DNA in rodents increases significantly with age (from 3 8-OHdG residues per 10^8 residues in young mice to 8 per 10^8 residues in the old ones) [7, 14, 17, 31, 42].

A significant number of works are dedicated to the comparison of the amounts of damaged DNA in mitochondrias and nucleus, as mitochondrial DNAs are considered to be the place of accumulation of errors [37, 38]. The process of DNA alkylation is considered to be rather dangerous – it can result in mutations and development of tumours. 3-methyladenine influences the process of replication, O^6-methylguanine and O^4-alkylthymine are considered to be of mutagenic origin, and 7-methylguanine is considered to be a relatively harmless compound (which can be explained by insufficient information on its specificities) [20, 22, 26].

However, the thought does exist that methylation of cytosine may be considered a norm (as one of epigenetic mechanisms). This issue is a topic for a dedicated discussion, which has a significant number of works devoted to [55-57]. The most common place for methylation in human adult somatic cells is CpG-regions (app. 70% of them are methylated), at the same time, so called, non-CpG-methylation, is observed in embryonic stem cells. At early stages of development (from cell fertilization to the stage of eight cells) eukaryotic genome is non-methylated. Starting from the stage of eight cells and to morula methylation *de novo* takes place. At the stage of blastula the processes of methylation, which provide epigenetic re-programming, are completed [55-57].

The issue of reparation is not the topic of current review, yet it has no be noted that the presence of systems of effective reparation of DNA damages proper provides the stable condition of the cell. The issue of reparation was reviewed in detail in numerous publications [26, 32].

The reparation of the bulk of DNA damages is related to the presence of base excision repair (BER) and nucleotide excision repair (NER) [20, 26]. The first type of repair is fast and relatively simple, at the same time it requires the presence of several groups of enzymes, namely, glykolases, endonucleases, exonucleases, polymerases, and lyases. It is worthy to be noticed that the elimination of damaged regions takes place in a short time – some data show that human lung epithelium cell is capable of getting rid of damaging modifications in 8–65 min (depending on the form of damage) [7]. BER is considered to be the main type of correction of errors, formed as a result of alkylation and oxidation of DNA. The defects of BER lead to genome instability and influence the cell cycle, which results in carcinogenesis or apoptosis [1, 26, 32].

NER provides neutralization of errors in the region of several nucleotides (elimination of cyclobutane

dimers, intermolecular cross-links, *etc*). This type of reparation requires more time and is conditioned by the activity of more than 20 different types of enzymes. NER can be divided into two subtypes, *i.e.* global genome repair and transcription-related reparation. Various diseases, *e.g.* xeroderma, trichothiodystrophy, Cockayne's syndrome, are often to occur during NER disorders [1, 7, 20, 26, 32].

The modifications, formed as a result of alkylation, are repaired with highly-specialised enzymes of alkyltransferases [26].

The repair of products of deaminization of nitrogenous bases is considered to be important as well. The transitions, which occur due to these damages, are revealed during hereditary diseases, placing this group of modifications among the most dangerous ones. The enzymes, capable of correcting the damages, caused by deaminization, are as follows: T:G DNA-glycosylase, uracyl-DNA- glycosylase *etc*. [20, 26].

Yet the part of damages may be corrected by direct elimination of chemical groups. Thus, the excision of O^6-methylguanine is possible with the enzyme of narrow substrate specificity – MGMT – O^6-methylguanine-DNA-methyltransferase [23, 26].

The correction of sites of lost bases is performed by AP-endonucleases [27].

Double strand damages of DNA are of extreme importance. These damages result in lethal consequences for the cell. Double strand DNA damages take place when the molecules are over-loaded with various damaged components, which happen to occur under the influence of a strong destructive agent, irradiation, for instance. These damages are very hard to be corrected [20, 26, 32].

The majority of DNA damages formed in the cell can be repaired. However, their over-accumulation may result in irreversible changes, lethal case in particular [1, 7, 26, 58, 59].

Finally we have to mention that the absence of clear quantitative, and sometimes qualitative, results brings up the necessity of detailed investigation on this problem. The selection of appropriate method, which includes the factors of the form of damage and the type of the investigated cells is the issue of special importance. Some forms of damages have not been studied well-enough due to their biochemical specificities, whereas some – due to their insignificant quantities in the cells [1–7]. It should be taken into account also that, as it follows from the common practice, the results, obtained during the investigation of the isolated DNA and of the genetic material from intact cells, might differ significantly. Besides, DNA damages, defined in the cells indeed are the part of balanced damageability of genome. Only a small number of works present the information on the damages in dynamics. All quantitative determinations of DNA modifications present their constant level of some sort. The process of accumulation of damages is the result of misbalance in damage/repair ratio [1, 7].

There is almost no doubt in regards to that fact that having obtaining data on the DNA condition, it is possible to make a conclusion on functional condition of the cell. The studies on the specificities of DNA damages will also provide better understanding of carcinogenesis and ageing.

Н. В. Скрипник, О. А. Маслова

Оксидативные повреждения ДНК

Резюме

Проанализированы некоторые характеристики основных типов оксидативных повреждений ДНК: модификации азотистых оснований и дезоксирибозы, одноцепочечные и двухцепочечные разрывы, апуриновые/апиримидиновые сайты, межвалентные взаимодействия ДНК с белками. Приведена химическая структура наиболее изученных форм оксидативных повреждений ДНК. Указаны самые распространенные генотоксические факторы (активные формы кислорода, свободные радикалы, алкилирующие агенты). Рассмотрены современные методики качественных и количественных исследований повреждений ДНК.

Ключевые слова: оксидативный стресс, повреждения ДНК, генотоксические агенты.

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