Analytics of oxidative stress markers in the early diagnosis of oxygen DNA damage

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Abstract

Under homeostatic conditions, an equilibrium state between amounts of free radicals formed and their scavenging is observed. Free radicals are destructive only when present in excess. Pathological changes within cells and tissues can result from a persistent excess of free radicals. Living organisms are increasingly exposed to oxidative stress, resulting in oxidative DNA modifications. One such modification is 8-hydroxy-2′-deoxyguanosine (8-OHdG). It is considered a biomarker of oxidative stress and oxidative DNA damage. It has been found both in physiological fluids and in cells. This paper presents methods found in the literature for determining 8-OHdG expression in various kinds of biological material – blood, urine or liver homogenates. Methods for determining the biomarker expression have been grouped into direct and indirect methods, and the various levels of 8-hydroxy-2′-deoxyguanosine that can be determined by the different techniques are presented. The basic pros and cons of the various techniques are also discussed.

Key words: ELISA, free radicals, HPLC, 8-hydroxy-2′-deoxyguanosine, 32P-postlabeling

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Under homeostatic conditions, a state of equilibrium between the amounts of free radicals formed and their scavenging is observed. Free radicals are destructive only when they are present in excess. In normal conditions, their presence is indispensable to the proper functioning of the body, and their production is under the strict control of enzymatic and non-enzymatic systems. Free radicals are involved in such processes as cell growth, proliferation, apoptosis and differentiation.[1–4]

Disruptions of cell homeostasis and shifts in the pro-oxidant/antioxidant equilibrium in the direction of oxidation reaction are called oxidative stress. This phenomenon is particularly dangerous for the respiratory system, cardiovascular system, brain and eyes. Pathological changes within cells and tissues can result when this state persists.[2–4] Figure 1 shows the 3 main effects of oxidative stress.

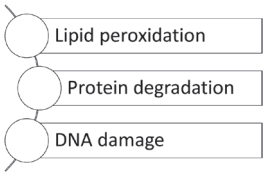

Fig. 1. The 3 main effects of oxidative stress

Lipid peroxidation

Lipid peroxidation is the most widely known biological free radical chain (FRC) reaction, entailing the oxidation of unsaturated fatty acids or other lipids comprising phospholipids, whose products are peroxides of these compounds. It is significant that peroxidation is not initiated by reactive oxygen species (ROS); the presence of ROS will only intensify the peroxidation process, not initiate it.

Like all FRC reactions, peroxidation can be divided into 3 stages:

- Initiation: The fatty acid radical is produced at this stage. Initiators in living cells are the radicals hydroxyl ($HO^{\cdot}$), peroxy ($LOO^{\cdot}$), alkoxy ($LO^{\cdot}$) and alkyl ($L^{\cdot}$), as well as ozone, sulfur dioxide and nitrogen dioxide. The separation of hydrogen leads to the formation of an alkyl radical.

$$LH \xrightarrow{HO^{\cdot}} L^{\cdot} + H_2O$$

- Prolongation: Volatile fatty acid radicals readily react with molecular oxygen, forming peroxides. These peroxides are characterized by a low level of stability and thus are capable of reacting with more fatty acid molecules, creating more radicals. It is a cyclical process.

$$L^{\cdot} + O_2 \longrightarrow LOO^{\cdot}$$
$$LOO^{\cdot} + LH \longrightarrow LOOH + L^{\cdot}$$

- Termination: When the concentration of free radicals is sufficiently high, the probability of collision between two radicals grows significantly. The collision of two radicals ends the process.

$$L^{\cdot} + L^{\cdot} \longrightarrow L - L$$
$$LOO^{\cdot} + LOO^{\cdot} \longrightarrow L = O + LOH + O_2$$
$$LOO^{\cdot} + L^{\cdot} \longrightarrow L = O + LOH$$

Dimers of fatty acids, hydroxy acids and oxoacids are products of the termination reaction. Often there is also a connection between peroxidation products and proteins present in the cell membranes, creating protein-lipid connections.

Products of lipid peroxidation may be subject to further changes, such as the β-elimination reaction, which produces, among other things, malonic dialdehyde (MDA) or 4-hydroxynonenal. Due to their small size, these products can easily diffuse through biological membranes and can therefore be the "secondary relay" of DNA damage caused by reactive oxygen species. The resulting aldehydes are cytotoxic, mutagenic and carcinogenic, and can cause rupture in DNA strands. The primary effects of lipid peroxidation also include the loss of activity of cell membranes, changes in their ability to inhibit the calcium pump, or weakening of the relation between electron transport in the respiratory chain and adenosine triphosphate (ATP) production.[2,5–7]

Protein degradation

Protein degradation is an inherent effect of aerobic cellular metabolism, and despite many defense mechanisms, it leads to the oxidation of biomolecules. When oxidative stress occurs, it is mainly the thiol groups (-SH) present in proteins that undergo the oxidation reaction. The reaction can be initiated by reactive oxygen species such as $O_2^{\cdot -}$, H_2O_2 or $HO^{\cdot}$. The products of these reactions are thiol radicals ($RS^{\cdot}$), which are readily dimerized to sulfides.

$$RSH + O_2^{\cdot -} + H^+ \longrightarrow RS^{\cdot} + H_2O_2$$
$$2RSH + H_2O_2 \longrightarrow RS^{\cdot} + 2H_2O$$
$$RSH + HO^{\cdot} \longrightarrow RS^{\cdot} + H_2O$$
$$2RS^{\cdot} \longrightarrow RSSR$$

Protein oxidation by ROS can occur in the polypeptide chain, or can involve amino acid residues; aromatic residues are among the most reactive amino acid residues. The oxidation of polypeptide chains is a similar process to lipid peroxidation, but the process is not a chain. An alkyl radical is a result of protons releasing (involving hydroxyl radicals) from the α-amino acid carbon. The alkyl radical is converted to alkyl hydroperoxide by reaction with oxygen. This product is capable of being converted to an alkoxy radical, a radical that activates the fragmentation of the polypeptide chain. Thiol damage leads to a loss of protein activity, changes in various kinds of enzymes and transporters, as well as breaches of calcium homeostasis. The oxidation of -SH groups in proteins leads to disintegration of membranes and changes in membrane permeability. The accumulation of oxidized protein products leads to cell dysfunction and, in critical situations, even to cell death.[2,8–10]

DNA damage

Damage to DNA by free radicals occurs much less frequently than oxygen damage to proteins and lipids. There are two theories that attempt to explain the cause of DNA damage. The first of them is that DNA damage is the result of a site-specific Fenton reaction (the generation of a hydroxyl radical in the reaction of transition metal ions [present in the DNA] with hydrogen peroxide).[11] According to the second theory, the effect of oxidative stress increases the intracellular concentration of calcium ions, which in turn activate nucleases digesting DNA.[12]

The hydroxyl radical is considered the main source of oxygen damage. Its interaction with DNA molecules can lead to the formation of single and double strand breaks of DNA cross-links or modifications in the nitrogen bases. Mitochondrial DNA (mtDNA) is more susceptible to oxygen damage than nuclear DNA. This may be explained by:

- the lack of nuclear proteins in mtDNA, which could protect it from damage,
- fewer repair opportunities, and/or
- the proximity of the respiratory chain.

DNA damage causes an increase in its mutagenic or immunogenic properties.[1,13]

Metabolic changes in cells resulting from oxidative stress include:

- reduction of the concentration of ATP in the cells. Such a reduction may be caused by damage to mitochondria; by deactivation of glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), which leads to inhibition of glycolysis reactions in the cells;, increased catabolism of adenine nucleotides; or increased consumption of ATP bound to the active transport of oxidized glutathione;
- increases in calcium ion concentration in the cytoplasm due to deactivation of the calcium pump;
- depolarization of the cell membrane, possibly due to deactivation of K, Ca, Na channels, resulting in increased cell membrane permeability;
- decreases in glutathione levels and reductions in the ratio of the reduced form of glutathione (GSH) to oxidized glutathione (GSSG). Another danger is the formation of glutathione in various connections with xenobiotics, the products of lipid peroxidation, or proteins present in the cell. Such products are disposed of outside the cell, and this process is associated with increased consumption of ATP, which also contributes to reductions in intracellular glutathione.[1,14]

8-hydroxy-2'-deoxyguanosine: A measure of oxidative damage to DNA

Guanine (G) is an aromatic heterocyclic compound, the construction of which is based on a fused pyrimidine and imidazole ring; it is therefore classified as a derivative of purine (guanine's systematic name is 2-amino-6-hydroxypurine). Guanine is a basic building block of both DNA and RNA. The nucleic acid forms a complementary pair with cytosine (C). Guanine, both in the free state and as a nucleoside, is particularly susceptible to the effects of free radicals in the C8 position, as shown in the diagram below (Fig. 2).

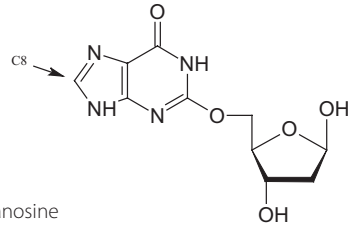

Fig. 2. Deoxyguanosine

Two of the entire pool of reactive oxygen species – hydroxyl radical ($HO^{\cdot}$) and singlet oxygen ($^1O_2$) – are capable of oxidative modification of guanine.

There are 2 theories regarding the mechanisms of how a hydroxyl radical attacks the guanine molecule. The first one assumes that the adduct of $HO^{\cdot}$ with the guanine C8 position is created directly, as shown in Fig. 3. The second hypothesis is that the process of oxidative damage to guanine has two steps. In the first stage of the process an electron from a molecule of guanine is abstracted with the participation of a hydroxyl radical (the abstraction of an electron can also be an effect of quantum radiation or some other equally strong attack, by an oxidant such as a ferrous radical[15], or as a result of the reaction of type I photosensitizers).[16–19] In the second stage of the reaction, the final product is formed (Fig. 4).

Oxidative damage to guanine (deoxyguanosine) may also occur as a result of the interaction of biomolecules with singlet oxygen.[20–24] As with the hydroxyl radical, the mechanism for this attack is explained by two equally plausible theories. In both cases, the creation of 8-oxoguanine proceeds to form an intermediate product: 4,8-endoperoxide (Fig. 5).

In the scientific literature, the names 8-hydroxyguanine and 8-hydroxy-2'-deoxyguanosine are used interchangeably, as they focus on the principle and the modified nu-

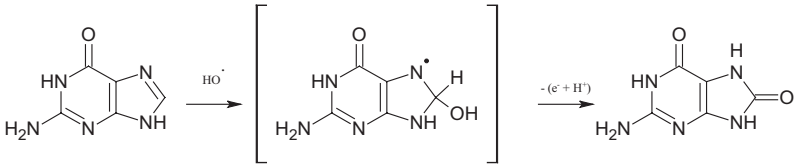

Fig. 3. One theory of the mechanics of a hydroxyl radical attacking guanine[15]

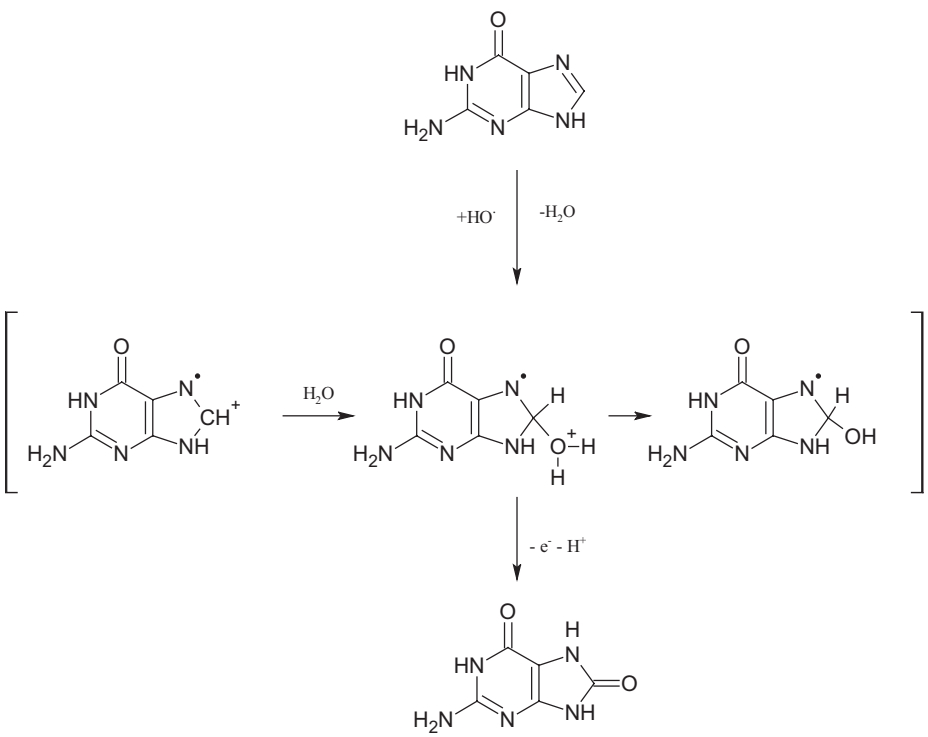

Fig. 4. Another theory of the mechanics of a hydroxyl radical attacking guanine[15,17]

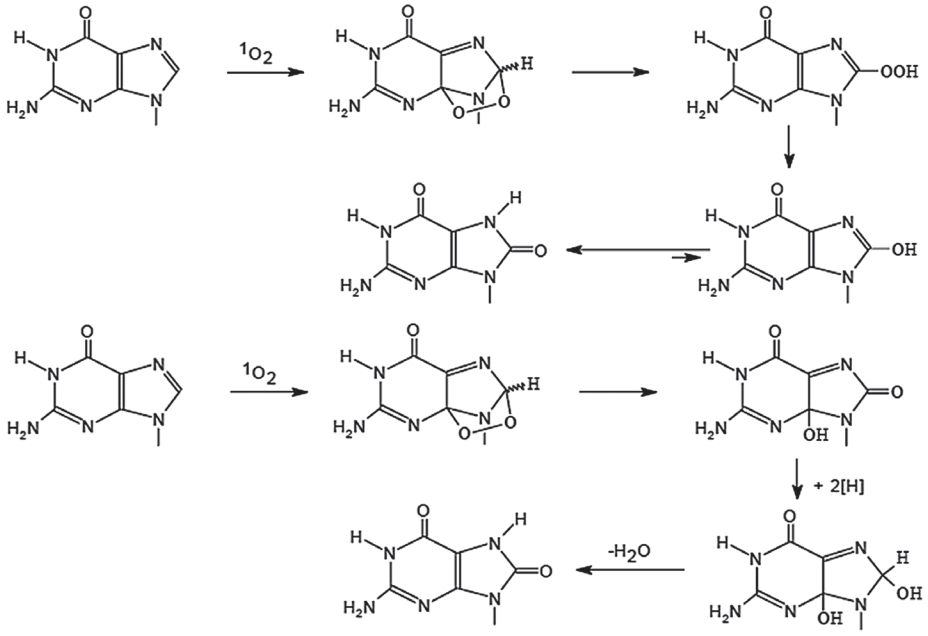

Fig. 5. The 2 mechanisms of single oxygen attack on guanine

Mechanism 1

Mechanism 2

cleotide. 8-hydroxy-2'-deoxyguanosine is subject to keto-enol tautomerism. Of the four possible tautomeric forms, the diketone form is the most favorable.[25] 8-hydroxy-2'-deoxyguanosine is the best known mutagenic modification of DNA. Studies have shown that the mutagenic potential of 8-OHdG results from an ability to disrupt the pairing process of DNA replication.[26–28] In DNA replication, defective 8-oxyguanine can form incorrect pairs with adenine. If the error is not corrected, transversion type GC → TA occurs in the next round of replication.

Numerous studies on the structure and thermodynamics of DNA duplexes containing 8-oxyguanine paired with adenine and cytosine, carried out mainly by nuclear magnetic resonance techniques and X-ray crystallography, have shown the relationship between the mutagenic properties of 8-OHdG and the structure of the resulting connections. Figure 6 shows two sets of connections, corresponding to 8-oxodG: dA and dC. 8-oxodG exists in 2 configurations: syn-periplanar (in conjunction with dA) and antiperiplanar (in combination with dC). The combination of 8-oxodG and dC is stabilized by 3 hydrogen bonds. However, the combination of 8-oxodG and dA is stabilized by two hydrogen bonds, so in this case it is possible to produce stable base pairs.[26,29–31]

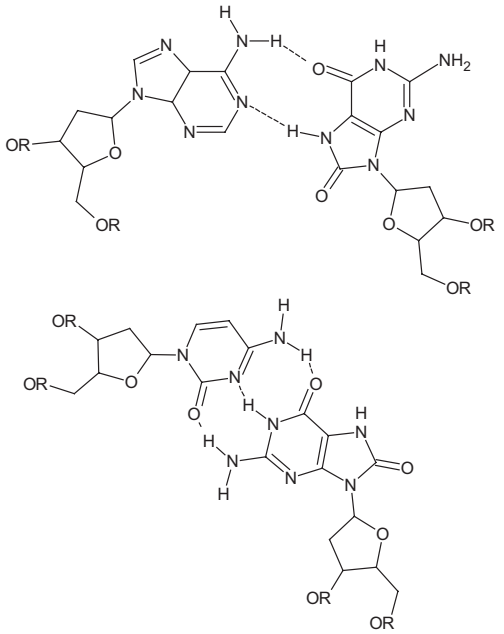

Fig. 6. A: 8-oxodG-dA; B: 8-oxodG-dC

The first mention in the literature of 8-OHdG as oxidative damage arising in vivo appeared in 1984.[32] Today, nearly 20 DNA adducts can be understood, but 8-OHdG is the one that attracts the greatest interest in the scientific community. It has been proved that 8-hydroxy-2'-deoxyguanosine can be considered a biomarker of aerobic cellular DNA damage and oxidative stress.[28,29,33,34]

In addition to its presence in cells and tissues, the modified purine base is also present in urine. It is believed that the presence of 8-hydroxy-2'-deoxyguanosine in urine reflects DNA repair processes taking place in the body.[31,32,35]

Correlations between the amount of 8-OHdG in urine with the amount in tissues in different pathogenic processes are still being investigated. Undoubtedly, the greatest interest is in the correlation between the amount of 8-hydroxy-2'-deoxyguanosine and cancer, which can be explained by the mutagenic properties of 8-OHdG – namely, its ability to disrupt pairing rules in DNA transversion. The result can be a malignant cell transformation.

Increased amounts of 8-OHdG also found in patients suffering from arteriosclerosis, diabetes, neurodegenerative disorders of the brain (Parkinson's disease, Alzheimer's disease) and autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus).[26,35–38]

Methods of measuring oxidative DNA damage

Analytical methods developed to measure oxidative DNA lesions can be classified in 2 groups: direct methods and indirect methods, also known as enzymatic methods (Fig. 7).

Indirect approaches Alkaline cell lysis using DNA N-glycosylases required	Direct approaches DNA extraction required

• Strand break detection
  • AE/FPg
  • Comet/FPg
• Antibodies
  • Fluorescence detection (ELISA)
  • Radioactive detection (RIA)
  • Immunohistochemistry
• Ligation-mediated polymerase chain reaction (LM-PCR)
	• ^{32}P-labeling – nucleotides analyzed
	• HPLC – ECD, HPLC – MS/MS – nucleosides analyzed
	• GC – MS – DNA bases analyzed after derivatization

Fig. 7. Direct and indirect methods for the determination of 8-OHdG

Typical direct methods for determining 8-hydroxy-2'-deoxyguanosine expression include:

- high-performance liquid chromatography with electrochemical detection (HPLC-ECD),
- high-performance liquid chromatography coupled with tandem mass spectroscopy (HPLC-MS/MS),
- ^{32}P-postlabeling.

Indirect methods for the determination of 8-OHdG include:

- the comet assay (single-cell gel electrophoresis),
- alkaline elution,
- immunoenzymatic tests (ELISA, RIA),
- methods based on polymerase chain reaction (PCR).

Direct methods

Direct methods are based on:

- isolation of cellular DNA,
- enzymatic or chemical hydrolysis of these isolates to nucleotides, nucleosides or free bases,
- chromatographic separation and determination of the analytes sought.

High performance liquid chromatography with electrochemical detection (HPLC-ECD) is a method that is considered highly selective. This method utilizes the fact that the modified purine base 8-hydroxy-2'-deoxyguanosine has a redox potential lower than the potential of substantially unmodified nucleosides. Only 8-OHdG is oxidized when the appropriate potential is used. The combination high of performance liquid chromatography with electrochemical detection multiplies the sensitivity of the process.[39]

This technique uses two types of electrochemical detectors: amperometric and coulometric. In these detectors, the working electrodes are primarily carbon electrodes; silver chloride electrodes are usually used as reference electrodes.[37,39–48]

Only the mobile phases with the ability to conduct electric current can be used with HPLC-ECD.

The preparation of samples for analysis using this technique depends on the biological material used. As

mentioned earlier, 8-OHdG is present in urine in its unchanged form. Therefore the preparation of a urine sample for analysis by HPLC-ECD for the 8-OHdG content relies heavily on solid-phase extraction (SPE) technology, which allows for significant enrichment of the analyte.[49–54]

If the analyte is 8-hydroxy-2'-deoxyguanosine – considered as a product of hydrolysis of cellular DNA – it is necessary to carry out hydrolysis of the DNA, and digestion with the appropriate enzymes: nuclease P1 and alkaline phosphatase. Nuclease P1 cuts the DNA, leading to the formation of the corresponding nucleosides. Alkaline phosphatase catalyzes the separation and release of the phosphate residues of 8-hydroxy-2'-deoxyguanosine. The final stage of the preparation of the samples is ultracentrifugation filters of more than 3000 Da, using specially adapted tubes for this purpose, stopping the hydrolysis products of DNA.[42,44,45,52,55]

The measure of oxidative damage of DNA is the ratio of 8-hydroxy-2'-deoxyguanosine (electrochemical detection) to 2'-deoxyguanosine (detection by UV radiation). Table 1 shows the chromatographic conditions used in the literature cited.

High performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) is also used for the determination of 8-OHdG. This approach can be used thanks to the development of the electrospray ionization technique, which made it possible to achieve the required sensitivity for identifying DNA adducts.[56]

Gas chromatography coupled with mass spectrometry (GC-MS) is also used for the determination of 8-hydroxy-2'-deoxyguanosine. The use of this method requires acid hydrolysis, which causes degradation of the DNA into free bases. Because gas chromatography can be used only for the determination of volatile analytes, it is necessary to transform 8-OHdG to its volatile derivatives; the tetramethylsilane (TMS) and tert-butyldimethylsilyl groups (TBDMS) are used for this. Derivatization is carried out at elevated temperatures (130°C) in order to obtain higher performance.[53]

Derivatization allows for the detection of DNA damage using the mass spectrometer, usually with at least two specific ions to ensure proper specificity determination. The mass spectrometer has greater versatility than electrochemical detectors and it can also be used for the determination of other types of DNA damage than 8-hydroxy-2'-deoxyguanosine.[54,57]

The ^{32}P-post-labeling method is another of the direct methods. This method is based on the enzymatic hydrolysis of DNA adducts that did not show radioactive properties to 3'-phosphonucleosides, then [^{32}P] phosphorylation of three -OH groups with [γ-32P] ATP and polynucleotide kinase.[58,59] Adducts are separated from unmodified nucleotides by thin-layer chromatography (TLC). This entails a two-way technology to develop the chromatograms. Chromatograms are read by audioradiograph.[58,59] The standard procedure has been modified in various ways over years of research, but only two of the enhancements have been of real interest to the research community (Figs. 8, 9):

- the use of nuclease P1 before the marking process – an enzyme that has the ability to dephosphorize only unmodified nitrogen bases. Nucleotides do not mark radioactive phosphorus. This behavior increases the sensitivity of the method;
- extraction of hydrophobic DNA adducts with n-butanol in the presence of a phase transfer agent: tert-butylammonium chloride. As a result, only the hydrophobic adducts are left in the organic phase, which is then subjected to isotopic labeling.[59]

Indirect methods

The principle of indirect methods (also known enzymatic methods) is based on the action of a specific enzyme – N-glycosylase DNA – which cuts oxidized nitrogen bases, causing a break, and DNA allowing for the detection of the breaks.[60]

Indirect methods used for the determination of 8-OHdG in biological tests include enzyme-linked immunosorbent assays (ELISAs), which are among the most widely used tests in biomedical research.[61,62]

The principle of operation is based on the formation of specific immune complexes between the antibody and

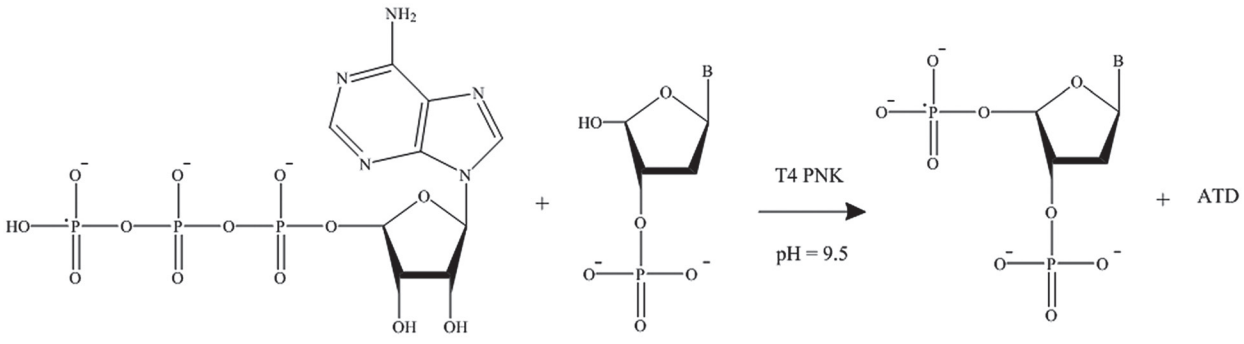

Fig. 8. Enzymatic hydrolysis of phosphorus and radioactive labeling of DNA adducts

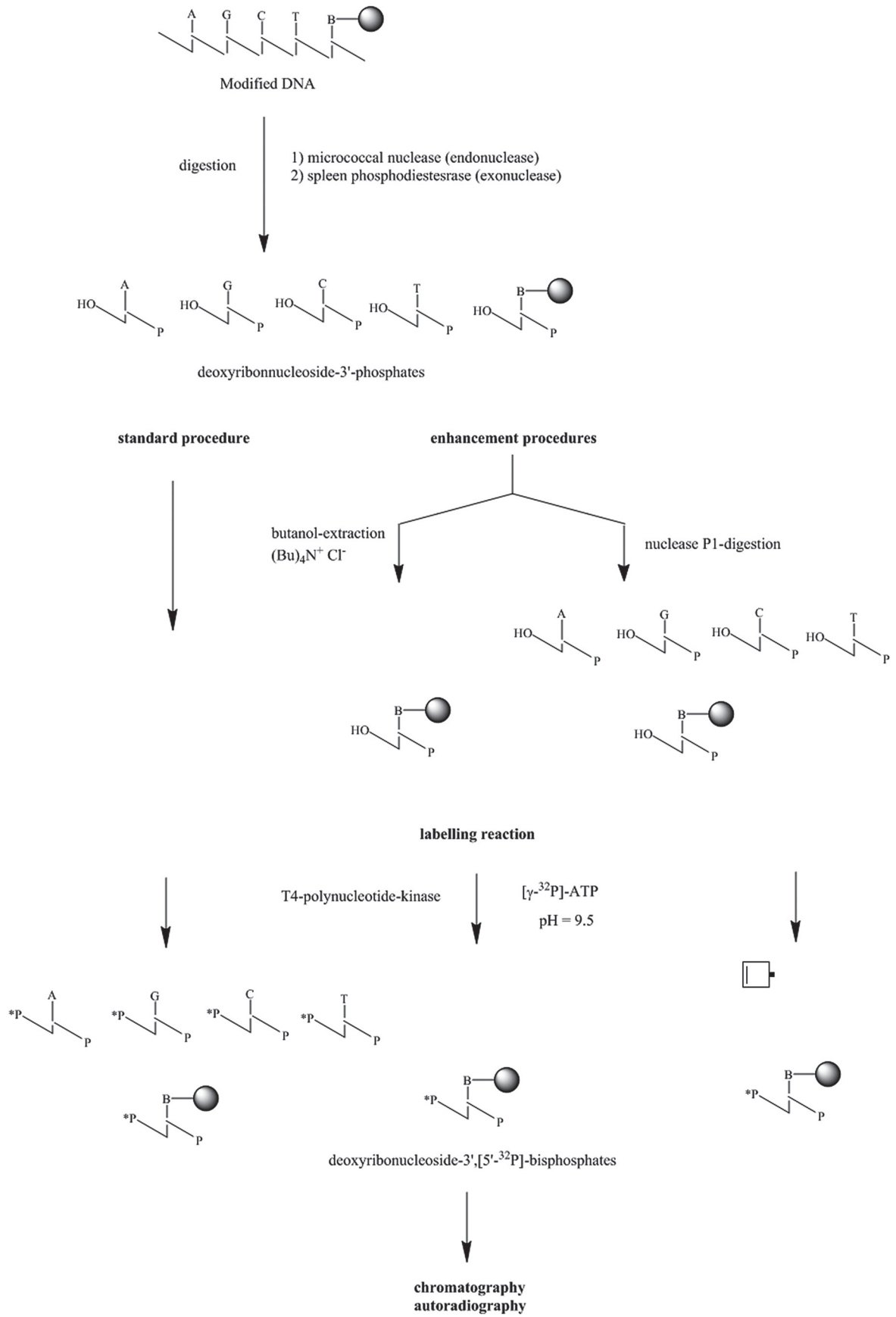

Fig. 9. Diagram of the ^{32}P-post-labeling process

Table 1. The summary of chromatographic methods for the assessment of the 8-OHdG in biological materials

Biological material	Stationary phase	Mobile phase	Detector	Temperature [°C]	Voltage [mV]	λ [nm]	Lit.
Urine	EcoCart, RP-18 125 × 3 mm 4 µm	50 mM KH2PO4 pH = 3.5 2.5% AcCN, 1 % MeOH 0.5 mL/min	amperometric detector working electrode: glassy carbon reference electrode: silver chloride	40.0	600	–	[69]
Urine, plasma, lymphocytes	ODS (Technicol, Stockport Cheshire, UK), 250 mm 5 µm	30 mM NaOH, 10 mM AcOH 41 mM AcONa, 12.5 mM citric acid (pH = 5.0), 10% MeOH 0.8 mL/min	amperometric detector working electrode: glassy carbon reference electrode: silver chloride	–	600	254	[37]
Urine	Asahipak ODP-50, C18 150 × 4.6 mm 5 µm	50 mM KH2PO4, 2 mM KCl, 2.5% AcCN 1 % MeOH, pH = 4.45 0.5 mL/min	amperometric detector working electrode: glassy carbon reference electrode: silver chloride	30.0	600	–	[39]
Urine	C18, 150 × 3.9 mm 10 µm	12.5 mM cytric acid, 25 mM AcONa, 30 mM NaOH, 20 mg/L EDTA 0.85 mL/min	amperometric detector working electrode: glassy carbon reference electrode: silver chloride	30.0	850	–	[40]
Urine	Eclipse XDB – C18 150 × 4.6 mm 5 µm	0–12 min: (10:90 v/v) 4–5 min: (50:50 v/v) MeOH, 10 mM HCOONa	amperometric detector working electrode: glassy carbon reference electrode: silver chloride	–	500	–	[41]
Cellular DNA, urine	C18 250 × 4.6 mm, 5 µm	10 mM NaH2PO4, 50 mg/L EDTA, 8% MeOH 1.0 mL/min	amperometric detector	32.0	550	245	[42]
Urine	YMC-Pack ODS-AM 250 × 4.6 mm	5% MeOH with 35 mM AcONa 12.5 mM citric acid pH = 7.5 0.8 mL/min	coulometric detector	25.0	350 – guard cell channel I – 150, channel II – 300	–	[43]
Urine	Supelcosil LC-18-S, Supelco 250 × 4.6 mm 5 µm	A: 50 mM KH2PO4 B: 50% CH3CN/MeOH 7:3 v/v 0–80 min 0–8 % B 80–85 min 8% B 85–90 min 8–50% B 90–100 min 50% B 100–105 min 50% B 1.0 mL/min	coulometric detector	–	guard cell – 400 channel I – 120 channel II – 400	–	[70]
Urine	EcoCart (Merck), C18 125 × 3 mm 4 µm	50 mM/l KH2PO4 (pH = 3.5) 1% MeOH 2.5% AcCN	amperometric detector, working electrode: glassy carbon reference electrode: silver chloride	–	600	–	[71]

Table 1. The summary of chromatographic methods for the assessment of the 8-OHdG in biological materials – cont.

Biological material	Stationary phase	Mobile phase	Detector	Temperature [°C]	Voltage [mV]	λ [nm]	Lit.
Blood, cellular DNA	Supelco, LC-18-S 250 × 4.6 mm	50 mM KH2PO4 2.5% AcCN pH = 5.5 1.0 mL/min	amperometric detector double carbon electrode	–	600	254	[45]
Lymphocytes	Spherisorb ODS-2, C18 250 × 4.6 mm 5 µm	20 mM AcNH4 (pH = 5.3) MeOH (85:15 v/v)	coulometric detector	–	450 – guard cell channel I – 130 channel II – 400	290	[46]
Monocytes	Supelcosil LC-18-S 150 × 3 mm 5 µm	10 mM KH2PO4 7.5% MeOH pH = 4.6 0.6 mL/min	coulometric detector	–	channel I – 50 channel II – 350	290	[72]
Lymphocytes	Supelcosil LC-18-S 250 × 4.6 mm	25 mM AcONa, 30 mM NaOH 10 mM AcOH 12.5 mM citric acid 1.0 mL/min	amperometric detector	–	850	260	[73]
Leukocytes	ODS-80Ts, TOSOH, Japan 150 × 4.6 mm	10 mM NaH2PO4 8% MeOH	coulometric detector	–	–	–	[47]
Lymphocytes, monocytes	Delta Pak, Waters, Miliford MA 150 × 3.9 mm 5 µm	20 mM AcONa MeOH (9:1 v/v), pH = 5.3 0.8 mL/min	coulometric detector	–	channel I – 200 channel II – 350	290	[74]
Leukocytes	Grom, Herrenberg – Kayh C18 250 × 4.6 mm	50 mM NaH2PO4 8% MeOH pH = 5.1 0.8 mL/min	amperometric detector	40.0	800	290	[75]
Liver homogenates	Supelcosil, C18 250 × 4.6 mm, 5 µm	50 mM KH2PO4 MeOH (90:10 v/v) pH = 5.5 1.0 mL/min	amperometric detector, carbon electrode	–	–	260	[48]
Liver homogenates	C18 250 × 4.6 mm 5 µm	50 mM/l KH2PO4 (pH = 5.5) H2O/ MeOH 85:15 v/v) 0.68 mL/min	coulometric detector	–	channel I – 20 channel II – 500	245	[76]
Liver homogenates	C18 150 × 4.6 mm 3 µm	50 mM AcONa 5% MeOH pH = 5.2	coulometric detector	–	channels I, II 120–890	–	[77]
Placenta tissue	–	50 mM/l KH2PO4 (pH = 5.5) MeOH (90:10 v/v) 1.0 mL/min	amperometric detector	–	–	–	[62]

the antigen. They are used to detect specific proteins using monoclonal or polyclonal antibodies conjugated with a suitable enzyme. ELISA tests have high sensitivity, repeatability and specificity, low implementation costs, and can be carried out quickly.

ELISAs are available in many configurations, as direct and indirect tests, sandwich ELISAs, competitive or inhibitory tests. For the determination of 8-OHdG in a competitive ELISA, capture antigens are used. These tests are performed using commercially available kits.[51,62–66]

The alkaline elution method is another indirect method. Before using this method, it is necessary to use formamidopyrimidine-DNA glycosylase (FPG), which creates single DNA strand breaks in the modified purine bases – the alkaline elution test is used for the detection of such breaks.

The procedure for this test involves lysis of cells in a highly alkaline environment, and the process of elution. The essence of this assay is to link the speed of elution to the degree of damage to the DNA – the faster rate of elution, the more damaged a DNA strand is.[60,67] The next indirect method, the use of which is also associated with FPg, is the comet method. In this method, the cells are deposited on an agarose gel on microscope slides and subjected to alkaline lysis, following which the sample is analyzed using electrophoresis. DNA migration towards the anode is possible only if there are breaks in the DNA.[60,63] Stained gels are analyzed by fluorescence microscopy. In cells whose DNA was damaged before lysis, broken threads are visible under the microscope as "comets". The percentage of cells with "tails" reflects the degree of DNA damage.[60,67,68]

Another indirect method for the determination of 8-hydroxy-2'-deoxyguanosine is ligation-mediated polymerase chain reaction (LM-PCR). This technique allows the determination of oxidative DNA damage and identifies where the gene is inactive.[60]

Conclusions

The methods described above are all the techniques reported in the literature for the determination of 8-OHdG in biological material. Fig. 10 shows a comparison of the different methods.

From the diagram it can be concluded that the indirect methods allow the determination of 0.1 to 0.2 defects per million unmodified nucleotides. This value is one of the lowest levels of oxygen damage to DNA that can be determined. Determination of high levels of oxygen defects is not possible by indirect methods due to their limited range of linearity. A method is considered to be quantitative when linearity of at least two orders of magnitude is observed.

The use of high performance liquid chromatography with electrochemical detection allows the determination of from 0.2 to 40 defects (8-OHdG) per million nucleotides. This method thus allows the measurement of the amount of DNA adducts at a level comparable to the indirect methods.

The amount of 8-hydroxy-2'-deoxyguanosine determined by gas chromatography combined with mass spectrometry is an order or two orders higher than that achieved by high performance liquid chromatography with electrochemical detection. This is probably due to the oxidation of DNA bases during the derivatization process, which takes place at elevated temperatures.

High performance liquid chromatography combined with tandem mass spectrometry gives results comparable to HPLC-EC, but with a much narrower range of linearity. Sample derivatization is not required when using HPLC-MS/MS and therefore potential errors in the process are eliminated.

The theoretical sensitivity of the ^{32}P post-labeling method is one in 100 million nucleotides. To determine this amount of DNA, very thorough preparation of DNA from isolates it is necessary before isotope labeling. This involves isolating a large amount of DNA. The labeling process is not very reproducible – different efficiency

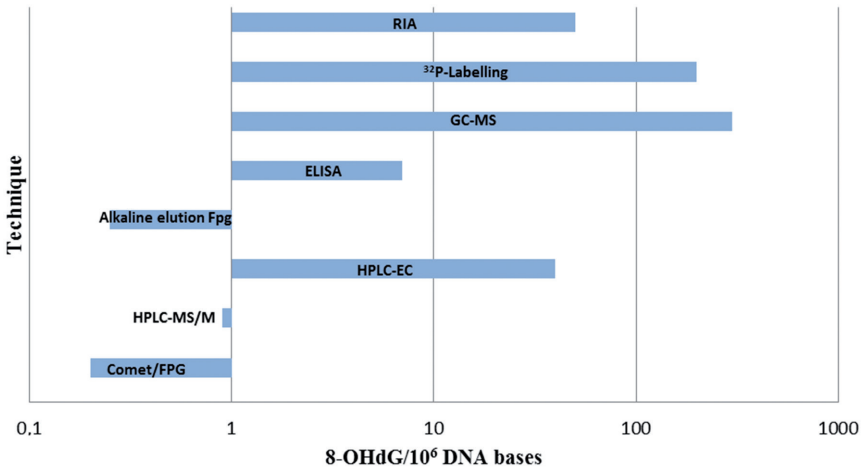

Fig. 10. The levels of 8-hydroxy-2'-deoxyguanosine that can be determined using various techniques

is achieved during each experiment, which can also be regarded as a drawback of this method. These disadvantages of this method can explain the results being higher than for other methods.

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