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Review Article

Measurement of urinary biomarkers of oxidative stress

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There is an increasing interest in the use of various biomarkers to assess the overall oxidative stress because of its implication in aging, cancer and several degenerative diseases. Urine is easy to collect and permits a non-invasive approach for measuring biological substances. In the past decades, urinary biomarkers of oxidative stress have been widely used both in research and clinical investigations and this also underscores the importance of having accurate and reliable assays. This article reviews the use of urinary biomarkers of oxidative stress (including oxidatively damaged DNA, oxidatively damaged RNA and the products of lipid peroxidation), and the pros and cons of the currently available assays for their measurement.

Key words: DNA, RNA, lipid, oxidative damage, urine

Oxidative stress

Growing scientific evidence indicates that oxidative stress is involved in many diseases, such as degenerative disorders (i.e., Parkinson's disease, Alzheimer's disease, diabetes and heart disease), cancer and aging[1,2]. Oxidative stress is defined as a state where the level of reactive oxygen species (ROS) exceeds the normal antioxidant capacity. Under oxidative stress, the excess levels of ROS could lead to the oxidation of important biomolecules, such as DNA, RNA, lipids and proteins, and may have implications for cellular function.

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Oxidatively damaged DNA in urine & its measurement

Oxidatively damaged DNA is perhaps the most studied in the literature. More than 30 nucleobase lesions have been identified[3]. 8-Oxo-7,8-dihydro-2'-deoxyguanosine (see Figure 1A) that was generated by hydroxyl radical, singlet oxygen, and one-electron oxidants in cellular DNA, has received great attention[4]. The detection of this lesion is considered important because of its abundance and mutagenic potential through G-to-T transversion mutations upon replication of DNA[5]. Oxidatively damaged DNA can be repaired, and the repair products are released into the bloodstream and consequently appear in the urine without further metabolism[6,7]. The modified base 8-oxo-7,8-

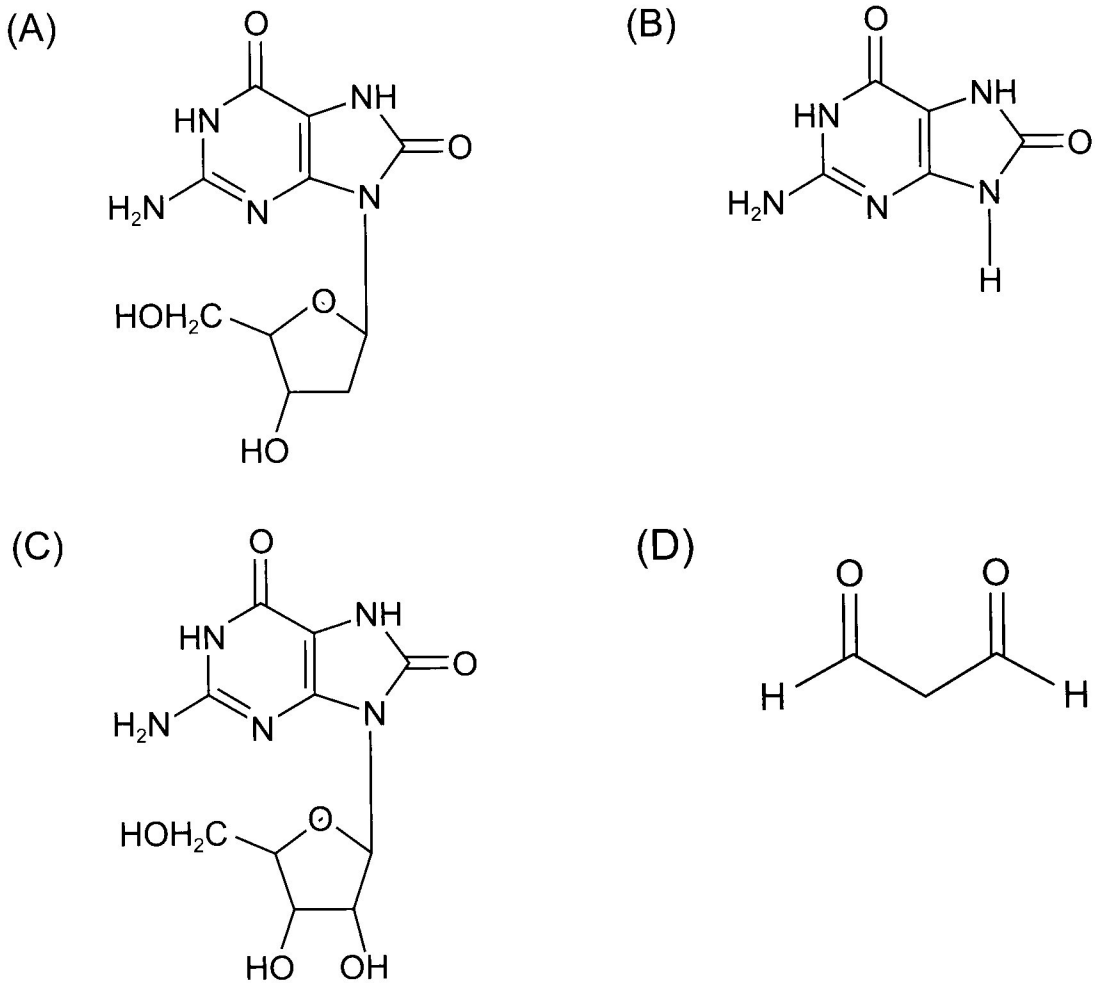

Fig. 1. Chemical structure of (A) 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), (B) 8-oxo-7,8-dihydroguanine (8-oxoGua), (C) 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and (D) malondialdehyde (MDA)

dihydroguanine (8-oxoGua, see Figure 1 B) and modified nucleoside (8-oxodG) present in urine represent the major repair products of oxidatively damaged DNA in vivo, presumably through the base excision repair (BER) and sanitization of the DNA precursor 2'-deoxyribonucleotide pool, respectively[8].

Various chromatographic-based methods have been developed for urinary 8-oxodG and 8-oxoGua quantification, including HPLC with electrochemical detection (HPLC-ECD), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem MS (LC-MS/MS). HPLC-ECD and GC-MS techniques have been established for over 10 years though they can be difficult to perform in the clinical laboratories because they are labor intensive and time-consuming (i.e., require chemical derivatization), or exhibit inadequate specificity when used to test urinary 8-oxodG and 8-oxoGua[6,9-11]. Alternatively, LC-MS/MS is a powerful technology that can overcome the sensitivity and selectivity issues in analysis of oxidatively damaged DNA. The possibility of using stable isotope-labeled internal standards in mass spectrometry has added greater reliability to the LC-MS/MS methods by accounting for the loss during sample work up and compensating for variability in MS detection. Furthermore, LC-MS/MS methods can be coupled with the online solid phase extraction (SPE)

system using column-switching device that allow us to prepare biological samples automatically[12]. Several LC-MS/MS methods have been developed for determining urinary 8-oxodG based on direct injection of microliter volumes of urine[12,13].

For immunoassay, competitive enzyme-linked immunosorbent assay (ELISA) has received widespread use in the world for 8-oxodG measurement alone (not for 8-oxoGua), probably because a number of commercial kits are available, which are relatively cheap and do not require specific technical skills or equipment. The benefits of ELISA include ease of use, no specialist equipment is needed and high-throughput. However, it is known that ELISA lacks specificity and overestimated the urinary level of 8-oxodG. The latest report by the European Standards Committee on Urinary (DNA) Lesion Analysis (ESCULA) clearly demonstrates that ELISA kits overestimated the urinary level of 8-oxodG approximately 2-6 times higher than those measured by chromatographic-based methods[14].

Oxidatively damaged RNA in urine & its measurement

RNA is more prone to oxidation than DNA because it is mostly single-stranded, its bases are not protected by specific proteins and its intracellular location is close to the ROS-generating mitochondria[15]. It is suggested that damaged RNA was mostly degraded and replaced by new synthesis. Damaged RNA could also be repaired by apurinic endonuclease[16] or other specific proteins[17] though the repair of RNA is thought to be a minor mechanism by which the cells deal with the oxidative RNA lesions[18]. Increasing evidence show that RNA oxidation is a contributing factor in certain diseases including hemochromatosis, cancer, diabetes, Alzheimer's disease and aging[15,19-22].

Like oxidation of DNA, the oxidation of RNA has been focused on the measurement of 8-hydroxylation product of guanine, the 8-oxo-7,8-dihydroguanosine (8-oxoGuo, see Figure 1C). The major approach for analysis of 8-oxoGuo in body fluids is the chromatographic-based techniques, such as HPLC with ECD or mass spectrometry. Though the former method usually required a complicated pretreatment (i.e., one-stage manual SPE or together with immunoaffinity column purification)[23,24]. Recently, LC-MS/MS methods have also been reported that are capable of being applied to the direct measurement of 8-oxoGuo in human urine[25,26].

Lipid peroxidation products in urine & its measurement

ROS can react with double bonds of polyunsaturated fatty acids (PUFAs) to yield lipid hydroperoxides. Malondialdehyde (MDA, see Figure 1D) is one of the major secondary oxidation products of peroxidized PUFAs. MDA has been shown to have mutagenic and cytotoxic effects and possibly to be involved in the pathogenesis of several human diseases, including atherosclerosis, neurodegenerative diseases, and cancer[27-29].

MDA is frequently determined spectrophotometrically as 2-thiobarbituric acid-reactive substances after its reaction with 2-thiobarbituric acid (TBA) in acidic medium at high temperature. However, the spectrophotometric approach has been seriously questioned for its lack of specificity because TBA reacts not only with MDA but also with many other compounds[30]. The use of HPLC-UV or fluorimetric detection has been therefore applied after the reaction of MDA with TBA to improve the selectivity and increase the sensitivity of the method[31,32]. Meanwhile, various derivatization agents (i.e., 2,4-dinitrophenylhydrazine and 2,3-diaminonaphthalene) were also adapted to the derivatization reaction of MDA to replace the TBA for decreasing the possible artifactual oxidation that was generated by TBA under high-temperature and acid conditions[31].

Mass spectrometric-based methods have also been developed for the measurement of urinary MDA. Shin and Jung[33] reported a GC-MS method following derivatization with penta-fluorophenylhydrazine for urinary MDA measurement. Recently, our research team

describes a simple LC-MS/MS method for fast determination of urinary MDA after derivatization with 2,4-dinitrophenylhydrazine[34].

Environmental, life style and other factors in relation to oxidative stress biomarkers

In addition to diseases, various factors could influence the urinary excretion of oxidative stress biomarkers. For example, cigarette smoking perhaps is one of the most studied lifestyle factors and many studies have shown that smokers have higher excretions of 8-oxodG, 8-oxoGua, 8-oxoGuo and MDA[25,35-37] owing to the cigarette smoke contains numerous ROS producing compounds. Environmental or occupational exposure of hazardous materials could also induce oxidative stress. Pollutants including heavy metals, polycyclic aromatic hydrocarbons, fine particulate matter (2.5 μm), black carbon, and carbon monoxide, etc., have been shown to increase the urinary 8-oxodG or MDA[34,38-41]. Personal characteristics, such as gender and age, have found to be associated with the urinary biomarker of oxidative stress. Males excreted higher concentration of 8-oxoGua than females and urinary levels of 8-oxoGua and 8-oxodG were found to be increased with age[20,37,42].

In the view of practical applications, spot urine sampling is the most frequently used method with creatinine to correct for variation in urine flow. Several studies reported that the variability of urinary 8-oxodG and 8-oxoGuo concentrations was reduced after normalization of the data based on the level of creatinine[14,43]. However, this could not be the case for 8-oxoGua. Recent studies have shown that the variability in the concentration of 8-oxoGua did not change after creatinine normalization, and using creatinine-corrected concentration of 8-oxoGua could have failed to provide the proper information of interest[7,44]. The reason for this is likely that 8-oxoGua does not follow the same transport mechanism of urinary excretion of creatinine.

Future perspectives

Clearly, there are a large number of pathological conditions in which oxidative stress may have a

Table 1. Urinary levels of oxidative stress biomarkers in healthy subjects and patients with various diseases in the literature.

DNA oxidation	Healthy controls	Patients	Methods	Ref.
8-oxodG	4.48 (n = 72)	6.19 (n = 56; cancer)	LC-GC-MS	[45]
(ng/mg creatinine)	4.76 (n = 32)	5.68 (n = 27; cancer)	LC-GC-MS	[46]
	651[a] (n = 134)	3696[a] (n = 222; cancer)	LC-GC-MS	[47]
	4.4 (n = 56)	7.2 (n = 59; amyotrophic lateral sclerosis)	LC-ECD	[48]
	3.3 (n = 27)	4.82 (n = 40; diabetes)	LC-MS/MS	[49]
	2.48 (n = 80)	3.07 (n = 168; bipolar disorder)	UPLC-MS/MS	[50]
	3.51 (n = 40)	4.26 (n = 40; schizophrenia)	UPLC-MS/MS	[51]
8-oxoGua	6.77 (n = 72)	12.2 (n = 56; cancer)	LC-GC-MS	[45]
(ng/mg creatinine)	10.4 (n = 32)	13 (n = 27; cancer)	LC-GC-MS	[46]
	1304[a] (n = 134)	2549[a] (n = 222; cancer)	LC-GC-MS	[47]
RNA oxidation				
8-oxoGuo	4.43 (n = 20)	12.1 (n = 21; hereditary hemochromatosis)	LC-MS/MS	[19]
(ng/mg creatinine)	3.41 (n = 80)	4.84 (n = 168; bipolar disorder)	UPLC-MS/MS	[50]
	4.76 (n = 40)	5.82 (n = 40; schizophrenia)	UPLC-MS/MS	[51]
Lipid peroxidation				
MDA	93.7 (n = 47)	533 (n = 47; cancer)	Spectrophotometry	[52]
(ng/mg creatinine)	117 (n = 27)	553 (n = 40; diabetes)	LC-UV	[49]
	60 (n = 10)	84 (n = 20; silica-induced lung diseases)	LC-MS/MS	[53]

[a] The value is expressed as ng/24 hr.

role. Urinary biomarkers of oxidative stress offer the means by which this stress could be monitored via a noninvasive route and in the meantime they may also have the potential to act as markers of disease development risk or assess efficacy of therapy. Therefore, to measure these biomarkers accurately and easily would be of great benefit to get a better understanding of the role of this damage in disease as well as to establish what levels of damage in urine are normal, or abnormal, for clinical application. Table 1 summaries the urinary levels of oxidative stress biomarkers in healthy subjects and patients with various diseases reported in the literature.

For sensitivity, selectivity and reliability, LC-MS/MS is certainly desirable. For high-throughput routine clinical use, LC-MS/MS is also applicable by coupling with a column-switching device. Despite its cost and specialty, LC-MS/MS may remain the most useful tool for measuring biomarkers of oxidative stress in urine.

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