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Urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG), a reliable oxidative stress marker in hypertension

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Abstract

The potential use of oxidative stress products as disease markers and progression is an important aspect of biomedical research. In the present study, the quantification of urine 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) concentration has been used to express the oxidation status of hypertensive subjects.

8-oxo-dG has been simultaneously isolated and assayed in nuclear (nDNA) and mitochondrial DNA (mtDNA). In addition, oxidative stress of mononuclear cells has been estimated by means of GSH and GSSG levels and GSSG/GSH ratio in hypertensive subjects before and after antihypertensive treatment. It is shown that oxidative stress decreases significantly in hypertensive patients after treatment the effect being accompanied by reduction of their blood pressure.

A significant correlation is observed comparing the yield of urine 8-oxo-dG and that isolated from mitochondria DNA. Moreover, urinary excretion of 8-oxo-dG also correlates with the GSSG/GSH ratio of cells. Conclusion: urine 8-oxo-dG assay is a good marker for monitoring oxidative stress changes in hypertensives.

Keywords: *Oxidative stress marker, hypertension, DNA damage, 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-oxo-dG), urine*

Introduction

Oxidative stress, an excessive production of reactive oxygen species (ROS) that outstrips antioxidant defence mechanisms, has been implicated in conditions that have an impact on the cardiovascular system; e.g. cigarette smoking, hypercholesterolemia, diabetes and hypertension [1,2]. The growing evidence for the importance of ROS creates the need for reliable and reproducible markers of oxidative stress, the assessment of which can be used to monitor treatment-induced changes. During the past two decades, the number of oxidative markers discovered has been growing [3]. Methods for the quantification of different types of oxidative stress indicators have been developed to reflect the oxidation products of the most important cell molecules including lipids, proteins, carbohydrates and nucleic acids [3–7]. This line of research still deserves attention in order to find accurate metabolites for clinical applications.

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One of the most characteristic ROS-induced byproducts is the oxidation of both nuclear (nDNA) and mitochondrial DNA (mtDNA). The most oxidation-prone among all the purine and pyrimidine bases is guanine [8]. Upon oxidation, a hydroxyl group is added to position C8 of the guanine molecule, resulting in the oxidative byproduct 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG), which is considered to be an important biomarker of generalized cellular oxidative stress and repair product [9]. In addition, due to its mutagenic potential, 8-oxo-dG creates marked genetic instability within the DNA structure [10]. Therefore, in the investigation of oxidative stress-related diseases, the assay of the oxidized moiety of guanine is a useful aspect. The 8-oxo-dG can be measured in cells and body fluids [11–13], although the significance of each of the measurements remains to be understood.

The present study was designed to assess the feasibility of using urinary 8-oxo-dG levels to express the degree of oxidative stress in hypertensive subjects. Simultaneously, these levels and those of 8-oxo-dG in mononuclear cells were assessed along with other oxidative stress parameters.

Material and methods

Subjects and methods

Patients included in the study were selected from an outpatient hypertension clinic. Those who fulfilled the inclusion criteria were invited to participate and informed written consent was requested. The following were the inclusion criteria: (a) essential hypertension defined according to the criteria of the VII Joint National Committee [14]; (b) aged 25–50 years; (c) WHO grade I–II; (d) glomerular filtration rate >60 ml/min; (e) never previously treated for hypertension or off medication for at least one month prior to the beginning of the study. Patients with diabetes mellitus, a fasting glucose in serum >120 mg/dl, with total cholesterol levels >240 mg/dl or cigarette consumption >10 per day were excluded. Control subjects were selected by age and weight, together with other clinical criteria. They were nonsmokers and free of disease and pathological antecedents. The Ethics Committee of the Hospital approved the study. A subgroup of patients underwent antihypertensive treatment with atenolol 50–100 mg during three months.

Blood pressure was measured using a mercury sphygmomanometer with the patient in the sitting position after 5 min of rest in a quiet environment, following the recommendations of the British Hypertension Society [15]. About 24 h ambulatory blood pressure monitoring was performed using an oscillometric monitor (Spacelabs 90202 or 90207) on a regular work day, following a standard protocol [16].

Mononuclear cell isolation and oxidative stress measurement

EDTA anticoagulated blood samples were obtained in the morning after a minimum of 8 h fasting. Whole blood (14 ml) was diluted with saline medium and mononuclear cells were isolated by Ficoll-Hypaque centrifugation [17] followed by three washes with the saline solution. The percentage of mononuclear cell suspension was 80–90%. Part of the isolated mononuclear cells was used for immediate analysis and the remainder was pelleted and preserved at -80°C to await DNA isolation.

Reduced glutathione (GSH) content of cells was determined using a previously described assay [18]. For the analysis of oxidized glutathione (GSSG), samples were treated with *N*-ethylmaleimide and bathophenanthroline disulfonic acid, derivatized and analyzed by high performance liquid columns (HPLC) as previously described [19]. The protein content was measured using the Bradford method.

Nuclear DNA isolation and digestion

nDNA was isolated following the Gupta method with the modification described by Muñiz et al. [18], in which chloroform isoamyl alcohol (24:1) is used instead of phenol for the removal of proteins. Isolated DNA was washed twice with 70% ethanol, dried and dissolved in 200 μl of 10 mM Tris/HCl, 0.1 mM EDTA, 100 mM NaCl, (pH 7.0) for its enzymatic digestion, as previously described [20]. In brief, 5 μg DNA/μl (total DNA 200 μg) was incubated with 100 units of DNase I in 40 μl Tris/HCl 10 mM and 10 μl of 0.5 M $MgCl_2$ (the final concentration of 20 mM) at 37°C for 1 h.

The pH of the reaction mixture was then lowered with 15 μl of sodium acetate 0.5 M (pH 5.1); 10 μl of nuclease P1 (5 units) and 30 μl of 10 mM $ZnSO_4$ were added (to give a final concentration of 1 mM) and the mixture was incubated for 1 h. After readjusting the pH with 100 μl of 0.4 M Tris/HCl (pH 7.8) followed by the addition of 20 μl alkaline phosphate (3 units), the samples were incubated for 30 min. Enzymes were precipitated with acetone (5 vol), removed by centrifugation and the supernatant evaporated to dryness.

Mitochondrial DNA isolation

Extraction of mtDNA was according to the method of Latorre et al. [21], with the modifications described below. Mononuclear cells isolated from an additional 14 ml whole blood sample, were resuspended in 300 μl PBS pH 7.4 and mixed with 300 μl of 10 mM Tris/60 mM NaCl/5% (wt/vol) sucrose, 10 mM EDTA, pH 7.8. About 400 μl of 1.25% $NaDodSO_4$/300 mM Tris/5% sucrose, 10 mM

EDTA (freshly mixed), pH 9.0, was then added. DNA extraction was performed using chloroform/isoamylalcohol 49:1 (v/v). After 10 min centrifugation at 13,000 rpm, the resulting supernatant was incubated for 30 min at 65°C and then 120 μl of 3 M potassium acetate buffer pH 4.8 was added. The mixture was then kept at −20°C for 20 min and followed by 10 min centrifugation at 13,000 rpm. Supernatant was transferred to an Eppendorf tube to which one volume of 2-propanol was added. The mixture was centrifuged as above and the obtained pellet resuspended in 150 μl Tris–EDTA 10 mM pH 7.8 and incubated for 30 min at 37°C. Then, 300 μl of 0.1% SDS, pH 12.5 were added and incubated at 65°C for 6 min, after which 300 μl of 3 M sodium acetate, pH 4.8 was added and the mixture was kept in −20°C for 20 min. The sample was further centrifuged and treated with 2-propanol as above. After a final centrifugation, the supernatant was discarded and the pellet was dried and resuspended in 35 μl of Tris–EDTA 10 mM, pH 7.8 and incubated for 60 min at 37°C previously to digestion.

The purity of the nDNA and mtDNA isolated using Latorre's method was determined by amplification of specific mtDNA and nDNA genes following the method described in [12]. We use the lipoprotein lipase gene, which is coded by nDNA, and the ATPase 8 gene, which is coded by mtDNA, to detect amplified fragments in the DNA preparations. All primers used in this study were designed using the Primer Express software (Applied Biosystems). Primer pairs specific for human ATPase 8 were (sense: 5′- GGCCCACC-ATAATTACCCCC-3′, antisense: 5′-TATGGGCTT-TGGTGAGGGAGG-3′. Amplification of the LPL gene (sense: 5′-TGGTCAGACTGGTGGAGCAG-3′, antisense: 5′-TGAGCGATTCAGGGCTTATGT-3′) served to check for nuclear genomic DNA contamination. PCR reactions were performed in a total volume of 10 μl containing 100 ng of either mtDNA or nDNA, 5 pmol sense and 5 pmol antisense primer, 0.2 mM of each dNTP, 1 U AmpliTaq Gold DNA polymerase (Applied Biosystems) using a 9800 Fast Thermal Cycler (Applied Biosystems). Amplification was performed as follows: 7 min at 98°C followed by 42 cycles at 94°C for 30 s, 60°C for 30 s, 72°C for 1 min followed by a final extension step at 72°C for 5 min. PCR products (5 μl out of 10 μl) were analyzed by agarose gel electrophoresis (2%) followed by ethidium bromide staining.

Assay of 8-oxo-7,8-dihydro-2′-deoxyguanosine

DNA hydrolysates were dissolved in high performance liquid chromatography (HPLC) grade water and filtered through a 0.2 μm syringe filter before applying the samples to a Waters ODS HPLC column (2.5 × 0.46 i.d; 5 μm particle size). The amount of 8-oxo-dG and dG in the DNA digest was measured by electrochemical and UV absorbance detection, respectively [22].

Chromatography

For the separation of 8-oxo-dG, a Waters 515 HPLC pump model was used. This separation was carried out using 5 μm Spherisorb ODS2 column (4.6 × 250 mm) with a flow rate of 1 ml/min. The running buffer for 8-oxo-dG from nDNA and mtDNA was 50 mmol/l potassium phosphate pH 5.1 in 5% of acetonitrile and the retention time was 7.5 min. Electrochemical detection of the urine samples was achieved with a ESA Coulochem II detector equipped with a 5011 high sensitivity analytical cell (sensibility of 1 μA). This is equipped with coloumetric (electrode 1) and amperometric (electrode 2) electrodes linked in series. For the purpose of this assay, the potentials set for the two electrodes were 0.2 and 0.4 V, respectively. The amount of 8-oxo-dG and deoxyguanosine (dG) in the DNA digest was measured by electrochemical and UV absorbance detection.

Urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine assay

The first urine in the morning was collected in polyethylene bottles. The volume of the sample was measured and after agitation, aliquots (2 × 1 ml) of the homogenized urine were kept at −80°C until further analysis.

The method of detection of 8-oxo-dG was based on that of Brown, RK et al. [23]. To 1 ml of urine, 100 μl of 3 mol/l Tris–EDTA solution pH 8.6 were added and vortex mixed for 30 s. The solution was then applied to a Bond Elute C18(OH)SPE (3 ml) column that had been prepared with 3 ml methanol and 3 ml water. The column was washed with 3 ml water followed by 3 ml 2.5% acetonitrile and 1.5% methanol in 10 mmol/l borate pH 7.9. The sample was eluted with 3 ml of the same buffer and applied to a Bond Elute strong cation exchange column (3 ml) prepared with 3 ml methanol and 3 ml borate buffer pH 7.9. The 8-oxo-dG was eluted with 2 ml of acetonitrile/-methanol buffer in borate and then adjusted to pH 6.0 with 1 mol/l HCl. About 4 ml of 50:50 dichloro-methane: propane-2-ol was added to the 2 ml of eluent and vortex mixed for 30 s. The sample was then centrifuged for 10 min at 3500 rpm, the upper aqueous layer aspirated off and 3 ml of organic layer evaporated to dryness under nitrogen at 50°C. Finally, the sample was reconstituted in 1 ml HPLC running buffer without acetonitrile and 50 μl injected into HPLC column. The 8-oxo-dG values were expressed as the ratio to creatinine urine concentration given in mmol/ml.

Chromatography

For urinary 8-oxo-dG, the running conditions were those described above, with the exceptions that the buffer used was 50 mmol/l potassium phosphate pH 5.1 in 5% of acetonitrile and the retention time was 7.5 min.

Statistical analysis

Values are presented as average and standard deviation or standard error. Comparisons between values at baseline or under antihypertensive treatment were sought by student *t* for paired. Pearson's correlation coefficients were used to assess relationships among variables. Multiple regression analysis was used to assess determinants of urinary 8-oxo-dG.

Results

To assess the optimization and accuracy of the HPLC-EC assay for the isolation and detection of 8-oxo-dG, appropriate chromatograms of both samples and standards were recorded at the beginning of each working day. Representative results are shown in Figures 1–3 where the chromatograms and retention times of 8-oxo-dG from urine, nDNA and mtDNA are compared with those of commercial standards.

Figure 1 represents the yields of 8-oxo-dG in the urine from a control (5.92 pmol) and a hypertensive subject (28.85 pmol) respectively.

As shown in Figure 2, the retention time of mtDNA 8-oxo-dG (0.30 pmol) corresponding to a hypertensive subject coincides with that of the standard (0.25 pmol). A total of 2800 μg/ml of mtDNA with a purity of 1.91 (260/280 nm) was obtained. After DNA enzymatic digestion, 40 μg were injected giving a final yield of 0.30 pmol.

In Figure 3, the retention times of nDNA 8-oxo-dG from a hypertensive subject is compared with that of a standard sample. Final concentration of nDNA was 4450 μg/ml with 1.8 (260/280 nm) of purity. As above, after its digestion, 40 μg were injected into HPLC and the resulting yield of 8-oxo-dG was 0.43 pmol.

The purity of the mtDNA was determined by measuring the presence of the lipoprotein lipase gene, which is coded by nDNA and the ATPase eight gene, which is coded by the mtDNA, in the DNA preparations. As show in Figure 4, a 313 bp segment of the lipoprotein lipase gene product was detected only in the nDNA from human mononuclear cells. The amplified segment of the ATPase 8 gene product (100 bp) was present in mtDNA from these cells.

The study was performed in 77 hypertensive subjects in absence of antihypertensive treatment. In 26 patients, the measurements were repeated after 3 months during antihypertensive treatment. Thus, a total of 103 paired samples of mononuclear cells and urine were collected for the analyses. The characteristics of the patients are shown in Table I. In mononuclear cells oxidative stress status, assessed by the levels of GSH and GSSG and DNA byproducts,

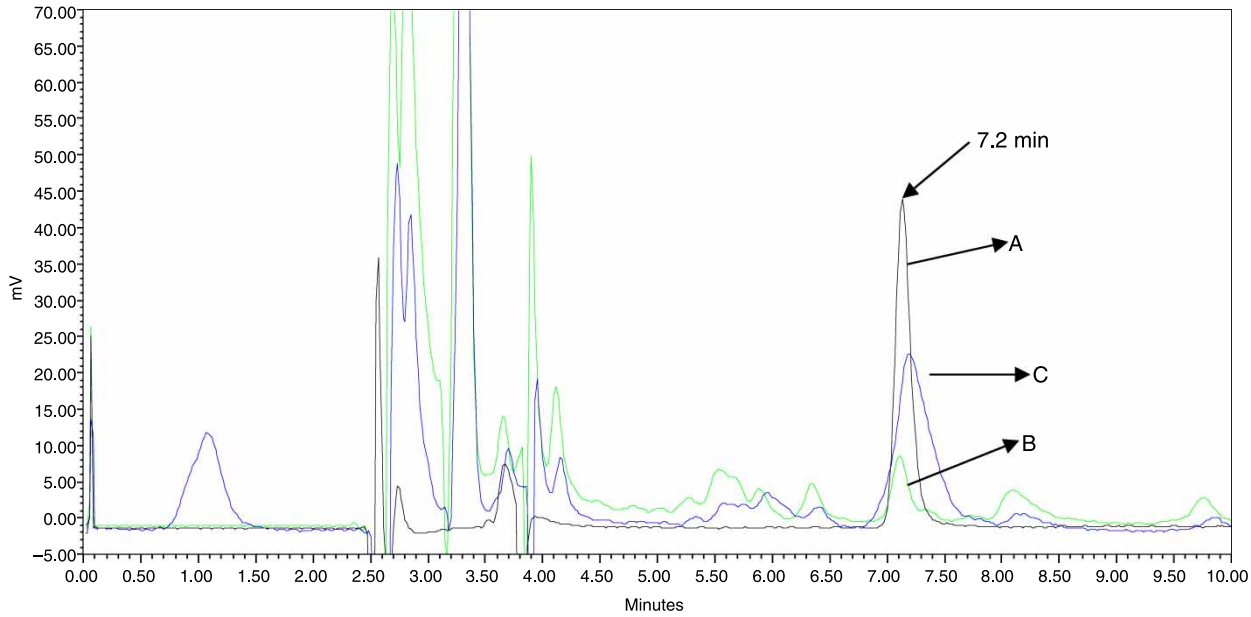

Figure 1. HPLC-EC chromatograms of 8-oxo-dG that separated from urine. Electrochemical responses of a standard 8-oxo-dG sample (40 pmol) and those isolated from urines with their retention times in minutes are shown. (A) standard samples 8-oxo-dG, (B) control subject (5.92 pmol), (C) hypertensive subject (28.85 pmol). About 50 μl of urine was used for 8-oxo-dG separation. The chromatographic conditions are given under Material and Methods.

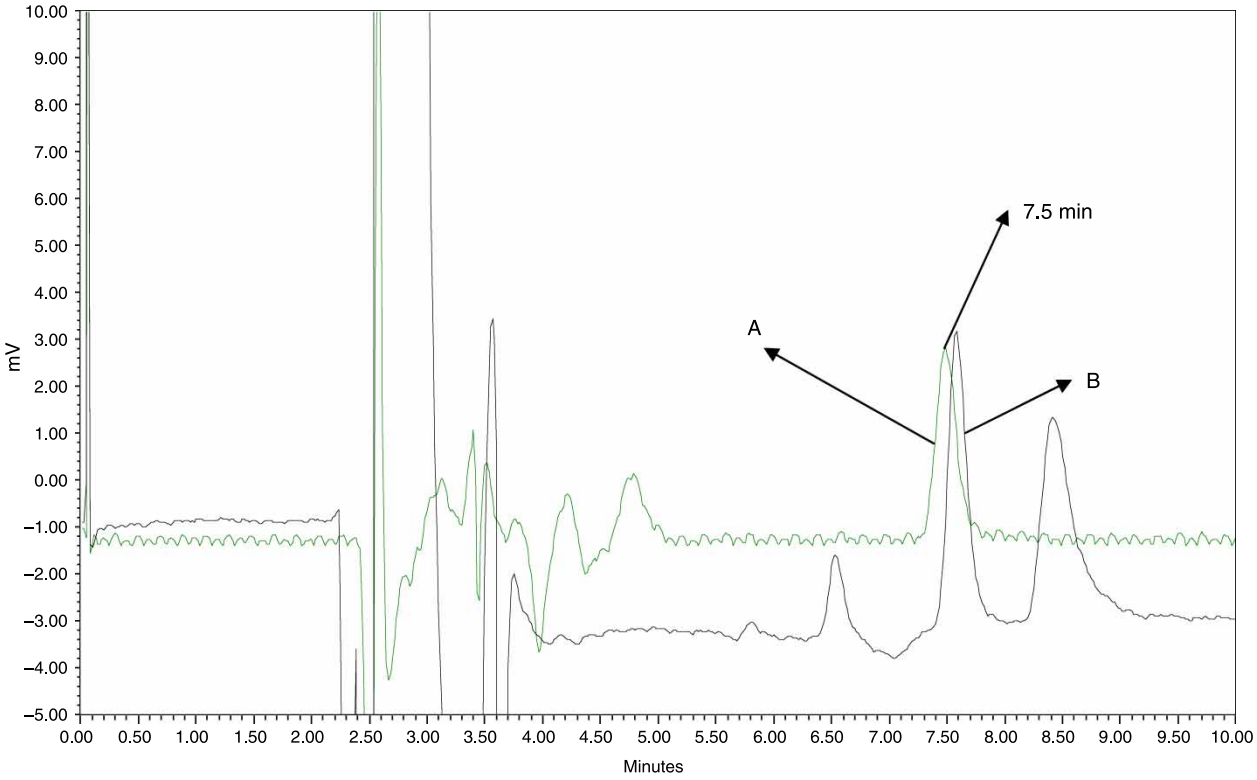

Figure 2. HPLC-EC detection of 8-oxo-dG isolated from mtDNA. A standard sample (0.25 pmol) of 8-oxo-dG in (A) is compared with that isolated from hypertensive mtDNA in (B). A representative result is shown and retention times of both samples are given. A total of 40 μg mtDNA were injected giving a yield of 0.3 pmol of 8-oxo-dG. Rest of the conditions as in Figure 1.

assessed by the proportion of 8-oxo-dG/dG from nDNA and mtDNA are shown in Table I. Simultaneously measured urinary levels of 8-oxo-dG expressed as a ratio of creatinine excretion are also shown in Table I.

In the 26 patients in whom the examination was performed, first in the absence of and later during antihypertensive treatment, a significant reduction for office (153.2 + 22.5 mmHg/96.4 + 18.1–133.4 + 19.0 mmHg/84.2 + 15.1 mmHg, $p < 0.001$, respectively) and for 24-hour ambulatory BP (136.2 + 18.2 mmHg/86.2 + 10.9–128.4 + 12.1 mmHg/78.2 + 9.2 mmHg, $p < 0.001$, respectively) was observed. Likewise, the reduction in oxidative stress status and byproducts in mononuclear cells were followed by a significant reduction in urinary 8-oxo-dG (Table II).

Urinary 8-oxo-dG was significantly correlated with oxidative stress status, GSH, GSSG, GSSG/GSH ratio, as well as with the 8-oxo-dG in mononuclear

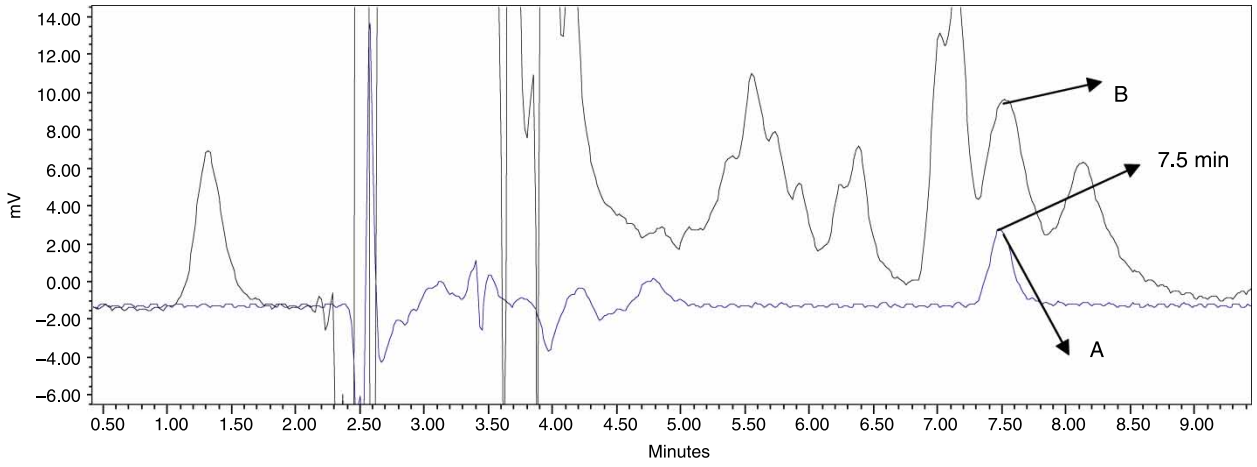

Figure 3. HPLC-EC detection of nDNA 8-oxo-dG. A standard sample (0.25 pmol) of 8-oxo-dG in (A) is compared with that isolated from hypertensive nDNA in (B). A total of 40 μg nDNA were injected giving a yield of 0.43 pmol of 8-oxo-dG. Other details as in Figure 2.

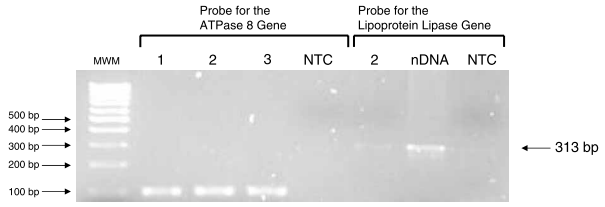

Figure 4. Characterization of nDNA and mtDNA isolated from human mononuclear cells. Amplification of ATPase 8 and lipoprotein lipase gene segments are shown. MWM: molecular weight markers. Lines 1, 2 and 3: amplification products from different mtDNA samples. NTC: no template negative control. nDNA: amplification product from nuclear DNA.

Table I. General characteristics of the study population.

Variables	Values
Number	61
Age (year)	43.7 ± 10.4
Sex (M/F)	40/21
Body mass index (kg/m^2)	27.3 ± 4.1
Office SBP (mmHg)	151.3 ± 20.4
Office DBP (mmHg)	95.7 ± 15.5
24-hour SBP (mmHg)	135.4 ± 17.2
24-hour DBP (mmHg)	85.3 ± 10.5
Baseline glucose (mg/dl)	99.3 ± 7.8
Total-cholesterol (mg/dl)	208.3 ± 30.1
HDL-cholesterol (mg/dl)	46.5 ± 9.1
Triglycerides (mg/dl)	129.0 ± 43.2
Oxidative stress parameters	
Mononuclear cells⋆	
GSH (nmol/mg prot.)	17.1 ± 0.54
GSSG (nmol/mg prot.)	0.93 ± 0.05
GSSG/GSH × 100	6.34 ± 0.46
8-oxo-dG nDNA	6.17 ± 0.15
8-oxo-dG mtDNA	6.69 ± 0.15
Urine⋆	
8-oxo-dG	3.09 ± 0.06

Values are mean ± standard deviation. 8-oxo-deoxyguanosine nuclear (8-oxo-dG nDNA) and mitochondrial (8-oxo-dG mtDNA) are expressed as the number of oxidized bases/10^6 dG. Urinary 8-oxo-dG is expressed as a ratio with creatinine (nmol 8-oxo-dG/mmol creatinine). GSH, reduced glutathione; GSSG, oxidized glutathione; ⋆ Values are mean ± standard error.

nDNA and mtDNA (Table III). The highest correlation coefficient between urine 8-oxo-dG release and glutathione metabolites was obtained with the GSSG/GSH ratio (0.63).

Finally, we analyzed whether the urinary 8-oxo-dG was more or less dependent on nuclear or mitochondrial sources by using a multiple regression analysis. Urinary 8-oxo-dG was significantly related to mitochondrial 8-oxo-dG ($p < 0.001$), but not to nuclear ($p = 0.886$). Mitochondrial 8-oxo-dG alone accounts for 28% of the variation of the urinary excretion.

Discussion

Essential hypertension is a heterogeneous disease with great variability on the individual risk for cardiovascular and renal damage. Treatment improves risk but the impact of treatment differs widely among individuals. Markers of risk help clinicians to decide about the individual risk of a given patient as well as the success or not of the therapy beyond the blood pressure reduction.

Although human hypertension is considered a state of oxidative stress that can contribute to the development of atherosclerosis and other hypertension—induced organ damage [2,24], the underlying mechanism remains to be fully clarified. It has been shown that superoxide radicals in and around vascular endothelial cells play a critical role in the pathogenesis of hypertension [25].

In addition to the role of endothelium, as a possible origin of ROS, circulating blood cells of differing nature have been implicated in hypertension-induced oxidative stress. These include activated T cells, macrophages, platelets and other superoxide producing cells. These cells contain substantial amounts of superoxide producing enzymes, mainly NADP(H) oxidase and xanthine oxidase whose expression and activities are significantly increased in hypertension. In addition, using different experimental models a close relationship between angiotensin II-induced

Table II. Oxidative stress byproducts in 16 control subjects and 26 hypertensives in absence and during antihypertensive treatment.

			Hypertension		
	Controls	p	Untreated	p⋆	Under Rx
Mononuclear cells					
GSH (nmol/mg prot.)	26.2 ± 0.24	<0.001	17.2 ± 1.33	<0.05	20.4 ± 2.28
GSSG (nmol/mg prot.)	0.16 ± 0.02	<0.001	0.67 ± 0.05	<0.05	0.53 ± 0.06
GSSG/GSH × 100	0.60 ± 0.07	<0.001	5.80 ± 0.64	<0.001	1.70 ± 0.10
8-oxo-dG nDNA	3.97 ± 0.65	<0.001	5.94 ± 0.24	<0.05	5.40 ± 0.26
8-oxo-dG mtDNA	5.40 ± 0.05	<0.001	6.65 ± 0.28	<0.05	6.20 ± 0.28
Urine					
8-oxo-dG	1.97 ± 0.05	<0.001	3.42 ± 0.10	<0.001	2.86 ± 0.09

Values are mean ± standard error. P values denote differences between control subjects and untreated hypertensives. P⋆ values denote differences between untreated and under antihypertensive treatment. 8-oxo-deoxyguanosine nuclear (8-oxo-dG nDNA) and mitochondrial (8-oxo-dG mtDNA) are expressed as the number of oxidized bases/10^6 dG. Urinary 8-oxo-dG is expressed as a ratio with creatinine (nmol 8-oxo-dG/mmol creatinine). GSH, reduced glutathione; GSSG, oxidized glutathione.

Table III. Pearson's correlation coefficients among 8-oxo-dG levels and glutathione metabolites in 103 paired samples.

	8-oxo-dG urine	8-oxo-dG nuclear	8-oxo-dG mitochondrial	GSH	GSSG	GSSG/GSH × 100
8-oxo-dG urine	–	0.51*	0.54*	− 0.59*	0.60*	0.63*
8-oxo-dG nuclear		–	0.67*	− 0.65*	0.57*	0.72*
8-oxo-dG mitochondrial			–	− 0.63*	0.62*	0.60*
GSH				–	− 0.75*	− 0.83*
GSSG					–	0.98*
GSSG/GSH × 100						–

Values are Pearson's correlation coefficients; * Values denotes significant correlation coefficient < 0.01.

hypertension and the activation of NADP(H) subunits, p47-phox, p22-phox and nox-2, has been established [26,27].

Endothelium is considered the target and mediator of both hypertension and oxidative stress which is, in turn, responsible for the hemodynamic and biochemical disturbances leading to atherosclerosis. Different oxygen-related metabolites and oxidation products have been proposed not only as useful biomarkers of oxidative stress in cardiovascular processes but also of tissue damage [4,24–29]. Oxidative stress status is also increased in the mononuclear cells of hypertensive patients where additionally, a significant correlation has been observed between the degrees of lipid peroxidation and DNA oxidation [28,30]. In previous studies, a good correlation of oxidative stress byproducts and tissue damage indicators has been observed. Thus, in microalbuminuric subjects the amount of GSH was significantly lower and the ratio GSSG/GSH was significantly higher that in the normoalbuminuric subjects [31].

In the present study, nuclear and mitochondrial 8-oxo-dG in mononuclear cells and urinary excretion of the damaged nucleoside, were measured simultaneously in hypertensive patients in the absence of and during, antihypertensive treatment. In these subjects, urinary excretion rates of 8-oxo-dG highly correlate not only with the 8-oxo-dG levels of mononuclear cells, but also with other estimates of oxidative stress such as GSH and GSSG. The correlation between urinary 8-oxo-dG and GSH, GSSG or GSSG/GSH was better than that obtained with genomic 8-oxo-dG and mitochondrial 8-oxo-dG with correlations coefficients of -0.59, 0.60, 0.63, 0.51 and 0.54, respectively. Nevertheless, a good correlation still exists between the yield of 8-oxo-dG in urine and the amount of both, nDNA and mtDNA, damaged bases in mononuclear cells of hypertensive subjects.

The biphasic distribution curve shown in Figure 5 represents the significant correlation coefficient obtained between GSSG/GSH values and urine of 8-oxodG. The release of 8-oxodG seems to stabilize arriving to a plateau. This effect could be probably attributed to the saturation of nucleoside transport systems.

The study was performed on hypertensive subjects with a normal glomerular filtration rate. In this population, a significant increment in oxidative stress and its reduction by antihypertensive treatment, has been described by our group and others [28,29,32]. Moreover, urinary 8-oxo-dG reflects the changes in oxidative stress induced by the antihypertensive treatment. Thus, in the present population of uncomplicated hypertensives, we were able to detect changes that may help us to monitor the potential beneficial effects of treatment by using a non-invasive sampling method.

Controversial results concerning whether or not urinary 8-oxo-dG represent the oxidative stress status of the body have been reported [33–35]. 8-oxo-dG is basically removed from DNA by base excision repair (BER) and is expected to be found in the urine as the base 8-oxo-7,8-dihydroguanine. Although nucleotide excision repair (NER) may lead to the production of 8-oxoGua-containing oligonucleotides and these fragments may be further hydrolysed to release 8-oxo-dG, this pathway has never been demonstrated. Olinski's group [34] suggests that 8-oxo-dG found in urine as the base rather than the nucleoside reflect DNA damage. At present, although the action of NER and subsequent digestion of oligonucleotide products could theoretically account for a portion of urinary

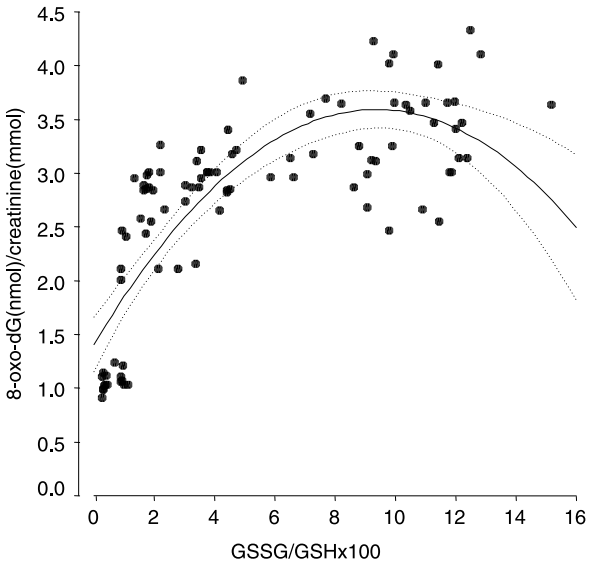

Figure 5. Relationship between GSSG/GSH in mononuclear cells and urinary 8-oxo-dG excretion.

8-oxodG, the pyrophosphohydrolase action of hMTH1 on 8-oxoGTP in cellular nucleotide pools would appear to be one of the most likely candidates for the generation of 8-oxodG as a repair product and its presence in urine [36,37].

Because oxidized nDNA in general undergoes repair, the repair products of the oxidative DNA lesions are fairly water-soluble and will be excreted into the urine without being further metabolized. These urinary oxidized products include free bases, ribonucleosides from RNA and deoxynucleosides and nucleotides from DNA. It should be emphasized that the excretion of the removed products into urine represents the average rate of damage and repair in the total body, whereas the level of oxidized base in nDNA is a measure of their concentration in the specific tissue/cells at the moment of sampling [38]. The values of 8-oxo-dG obtained are similar to those reported by other groups considering the differences of the methodology used [39–41].

Hypertension is a generalized process affecting a large number of cellular systems not only those located in the vascular walls but also to the large mass of blood circulating cells. Thus, it is expected that the whole body oxidative stress burden in hypertension is higher than in other more localized diseases. Furthermore, the data from the present study demonstrated a dependent relationship between urinary 8-oxo-dG and mtDNA 8-oxo-dG and less with nDNA. This can be explained considering that DNA in mitochondria are likely to be more susceptible to ROS-induced oxidative damage as oxygen metabolism is high and then the repairing mechanisms of mtDNA are much more active [8]. 8-oxo-dG will not be a diagnostic tool for hypertension but may give information about the level of risk mainly during antihypertensive treatment. Although initially proposed as a possible sources of urinary DNA lesions, recent research work with rats and human volunteers demonstrates that diet nor cell death appear to contribute to urinary levels of 8-oxodG [37].

In conclusion, urinary 8-oxo-dG emerges as a marker of oxidative stress in patients with essential hypertension in which a generalized oxidative stress is observed not only along the vascular walls, but also in circulating blood cells. Both the easy collection, which permits repeated measurements, as well as the discriminative power of the values, creates the opportunity for monitoring oxidative stress changes by measuring 8-oxo-dG in the urine samples of hypertensive patients.

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References

[1] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 3rd ed. Oxford, UK: Oxford University Press; 1999.
[2] Romero JC, Reckelhoff JF. Role of angiotensin and oxidative stress in essential hypertension. Hypertension 1999;34:943–949.
[3] Griffiths G. Biomarkers. Mol Aspects Med 2002;23:101–208.
[4] Vassalle C, Botto N, Andreassi G, Berti S, Beiagini A. Evidence for enhanced 8-isoprostane plasma levels, as an index of oxidative stress *in vivo*, in patients with coronary artery disease. Coron Artery Dis 2003;14:213–218.
[5] Minuz P, Andrioli G, Degan D, Gaino S, Ortalani R, Tommasolim R, Zuliani V, Lechi A, Lechi C. The F2-isoprostane 8-epiprotanglandin F2alpha increases platelet adhesion and reduces the antiadhesive and antiaggregatory effects of NO. Arterioscler Thromb Vasc Biol 1998;18:1248–1256.
[6] Chen KC, Achill SS, Kasai DS, Nishimura S, Loeb LA. 8-hydroxyguanine, an abundant form of oxidative DNA damage, causes G–T to A–C susbstitutions. J Biol Chem 1992;267:166–172.
[7] Gedik CM, Collins A. ESCODD (European standard committee on oxidative DNA damage) Establishing the background level of base oxidation in human lymphocyte DNA: Results of an interlaboratory validation study. FASEB J 2005;19(1):82–84.
[8] Nakabeppu Y, Tsuchimoto D, Ichinoe A, Ohno M, Ide Y, Hirano S, Yoshimura D, Tominaga Y, Furuichi M, Sakumi K. Biological significance of the defense mechanisms against oxidative damage in nucleic acids caused by reactive oxygen species. From mitochondria to nuclei. Ann NY Acad Sci 2004;1011:101–111.
[9] Cooke MS, Evans MD, Herbert KE, Lunec J. Urinary 8-oxo-2′-deoxyguanosine- source, significance and supplements. Free Radic Res 2000;32:381–397.
[10] Oliva MR, Ripoll F, Muñiz P, Iradi A, Trullenque R, Valls V, Drehmer E, Sáez GT. Genetic alterations and oxidative metabolism in sporadic colorectal tumors from a Spanish community. Mol Carcinog 1997;18:232–243.
[11] Tormos C, Chaves FJ, García MJ, Garrido F, Jover R, O'Connor JE, Iradi A, Oltra A, Oliva MR, Sáez GT. Role of glutathione in the induction of apoptosis and c-fos and c-jun mRNAs by oxidative stress in tumor cells. Cancer Lett 2004;208:103–113.
[12] Hamilton ML, Guo Z, Fuller CD, Van Remmen H, Ward WF, Austad SN, Troyer DA, Thompson I, Richardson A. A reliable assessment of 8-oxo-2-deoxyguanosine levels in nuclear and mitochondrial DNA using the sodium iodide method to isolate DNA. Nucleic Acids Res 2001;29:2117–2126.
[13] Topp H, Armbrust S, Lengger C, Schoch G, Davies J, Stichler W, Manz F, Fusch C. Renal excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine, degradation rates of RNA and metabolic rate in humans. Arch Biochem Biophys 2002;403:31–37.
[14] Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., Jones DW, Materson BJ, Oparil S, Wright JT, Jr, Roccella EJ. National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The JNC 7 report. JAMA 2003;289:2560–2572.

[15] Petrie JC, O'Brien ET, Littler WA, De Swiet M. British Hypertension Society Recommendations on blood pressure measurement. Br Med J 1986;293:611–615.
[16] Redon J, Miralles A, Liao Y, Lozano JV, Pascual JM, Cooper RS. Circadian variability and microalbuminuria in essential hypertension. J Hypertens 1994;12:947–954.
[17] Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Scan J Clin Lab Invest 1968;21:77–89.
[18] Muñiz P, Valls V, Perez-Broseta C, Iradi A, Climent JV, Oliva MR, Sáez GT. The role of 8-hydroxy-2′-deoxyguanosine in rifamycin-induced DNA damage. Free Radic Biol Med 1995;18:747–755.
[19] Asensi MD, Sastre J, Pallardó FV, García de la Asunción J, Estrela JM, Viña J. A high-performance liquid chromatography method for measurement of oxidized glutathione in biological samples. Anal Biochem 1994;217:323–328.
[20] Frenkel K, Zong Z, Wei H, Karkiszka J, Patel U, Rashid K, Georgescu M, Solomon JJ. Quantitative high performance liquid chromatography analysis of DNA oxidized *in vitro* and *in vivo*. Anal Biochem 1991;196:126–136.
[21] Latorre A, Moya A, Ayala FJ. Evolution of mitochondrial DNA in *Drosophila subobscura*. Proc Natl Acad Sci USA 1986;83:8649–8653.
[22] Wei H, Frenkel K. Suppression of tumor promoter-induced oxidative events and DNA damage *in vivo* by sarcophytol A: A possible mechanism of antipromotion. Cancer Res 1992;52:2298–2303.
[23] Brown RK, McBurney A, Lunec J, Kelly FJ. Oxidative damage to DNA in patients with cystic fibrosis. Free Radic Biol Med 1995;18:801–806.
[24] Raij L. Nitric oxide in hypertension: Relationship with renal injury and left ventricular hypertrophy. Hypertension 1998;189–193.
[25] Nakazono K, Watanabe N, Matsuno K, Sasaki J, Inoue M. Does superoxide underlie the pathogenesis of hypertension? Proc Natl Acad Sci USA 1991;88:10045–10048.
[26] Wallwork CJ, Parks DA, Schmid-Schonbein GW. Xanthine oxidase activity in the dexametasone-induced hypertensive rat. Microvasc Res 2003;66(1):30–37.
[27] Cifuentes ME, Pagano PJ. Targeting reactive oxygen species in hypertension. Curr Opin Nephrol Hypertens 2006;15(2):179–186.
[28] Redon J, Oliva MR, Tormos C, Giner V, Chaves J, Iradi A, Saez GT. Antioxidant activities and oxidative stress byproducts in human hypertension. Hypertension 2003;41:1096–1101.
[29] Blankenberg S, Rupprecht HJ, Bickel Ch, Torzewski M, Hafner G, Tiret L, Smieja M, Cambien F, Meyer J, Lackner KJ. AtheroGene Investigators Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. N Engl J Med 2003;349:1605–1613.
[30] Lenton KJ, Therrialut H, Fulop H, Payette H, Wagner JR. Glutathione and ascorbate are negatively correlated with oxidative DNA damage in human lymphocytes. Carcinogenesis 1999;4:607–613.
[31] Giner V, Tormos C, Chaves FJ, Saez G, Redon J. Microalbuminuria and oxidative stress in essential hypertension. J Intern Med 2004;255:588–594.
[32] Saez GT, Tormos C, Giner V, Chaves FJ, Lozano JV, Iradi A, Redon J. Factors related to the impact of antihypertensive treatment in antioxidant activities and oxidative stress byproducts in human hypertension. Am J Hypertens 2004;17:809–816.
[33] Foksinski M, Gackowski D, Rozalski R, Olinski R. Cellular level of 8-oxo-2′-deoxyguanosine in DNA does not correlate with urinary excretion of the modified base/nucleoside. Acta Biochim Pol 2003;50:549–553.
[34] Lunec J, Holloway KA, Cooke MS, Faux S, Griffiths HR, Evans MD. Urinary 8-oxo2′-deoxyguanosine: Redox regulation of DNA repair *in vivo*? Free Radic Biol Med 2002;33:875–885.
[35] Gackowski D, Rozalski R, Roszkowski K, Jawien A, Foksinski M, Olinski R. 8-oxo-7,8-dihydroguanine and 8-oxo-7,8-dihydro-2′-deoxyguanosine levels in human urine do not depend on diet. Free Radic Res 2001;35:825–832.
[36] Hayakawa H, Taketomi A, Sakimi K, Kuwano M, Sekiguchi M. Generation and elimination of 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-triphosphate, a mutagenic substrate for DNA synthesis, in human cells. Biochemistry 1995;34:89–95.
[37] Cooke MS, Evans MD, Dove R, Rozallski R, Gackowski D, Siomek A, Lunec J, Olinski R. DNA repair is responsible for the presence of oxidatively damaged DNA lesions in urine. Mutat Res 2005;574:58–66.
[38] Cooke MS, Evans MD, Burd RM, Patel K, Barmad A, Lunec J, Hutchinson PE. Induction and excretion of ultraviolet-induced 8-oxo-2′-deoxyguanosine and thymine dimmers *in vivo*: Implications for PUVA. J Invest Dermatol 2001;116:281–285.
[39] Loft S, Pousen HE, Vistisen K, Knudsen LE. Increased urinary excretion of 8-oxo-2′-deoxyguanosine, a biomarker of oxidative DNA damage, in urban bus drivers. Mutat Res 1999;441:11–19.
[40] Lenger C, Schöch G, Topp H. A high-performance liquid chromatographic method for the determination of 8-oxo-7,8-2′-deoxyguanosine in urine from man and rat. Anal Biochem 2000;287:65–72.
[41] Weiman A, Belling D, Poulsen H. Quantification of 8-oxo-guanine and guanine as the nucleobase, nucleoside and deoxynucleoside forms in human urine by high-performance liquid chromatography-electrospray mass spectrometry. Nucleic Acid Res 2002;30:4–8.