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ORIGINAL RESEARCH

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Oxidative damage on nucleobases and Hoehn–Yahr stage in Parkinson's disease

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ABSTRACT

Background: The imbalance between reactive oxygen species and the antioxidant barrier is a typical condition of Parkinson's disease (PD), and maybe the cause of oxidative alterations on DNA. The oxidation of nucleobase guanine (Gua) has been previously widely studied and the measurements of the hydroxylated form 8-hydroxy-Gua (8-OHGua) and its nucleoside 8-hydroxy-2-deoxy-guanosine (8-OHdG) have been proposed as sensible and reliable markers of this imbalance.

Objective: The study was carried out to demonstrate the relations between 8-OHGua, 8-OHdG, and the corresponding non-oxidized nucleoside 2-deoxy-guanosine (2-dG) with PD clinical progression.

Method: The measurements of 8-OHGua, 8-OHdG, and 2-dG were performed in urine samples of 198 PD subjects, ranked according to the Hoehn–Yahr scale (H–Y). The same analysis was also performed in a group of 33 subjects with other parkinsonisms.

Results: All markers were analyzed by using a high-performance liquid chromatography and electrochemical detection. For 8-OHGua, a new method was specifically optimized; the low limit of detection (10 ng/l) and good intra- and inter-assay coefficients of variation (2.2% and 3.6%, respectively), allowed reliable measurements of 8-OHGua in a wide range of concentrations (from 0.5 to 2,000 µg/l). Compared to the initial stages (H–Y < 2), 8-OHdG doubles in advanced ones (H–Y > 2) and 2-dG halves, both with statistical significance ($p < 0.001$). Furthermore, 8-OHdG increased more in males than in females, and its level was higher in parkinsonism than in PD. Even the values of 8-OHGua increased with the aggravation of the pathology, but in a less marked way and without statistical significance.

Conclusions: The redox imbalance in PD causes oxidative damage on DNA that increases with the progression of the disease. By attributing to 8-OHdG the role of marker of extent of oxidative damage, to 8-OHGua the one of efficiency of removal of oxidized bases from DNA, and to 2-dG the one of recovery of native nucleosides for the replacement of oxidized ones, our data seem to show that the DNA repair mechanisms in PD are insufficient to counteract the chronic oxidative insult that characterizes the disease.

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Introduction

The oxidative stress, considered as the imbalance of physiological equilibrium between the production of reactive oxygen species (ROS) and their metabolic neutralization, is a common condition in neurodegenerative diseases. Particularly, in Parkinson's disease (PD), the oxidative alterations affect the dopaminergic neurons of the mesencephalic substantia nigra. Although it is unlikely that oxidative imbalance represents the primary event of the degenerative process, it is certain that it participates in a substantial way to the progression of damage [1].

Under redox imbalance conditions, the free radicals overproduction can cause oxidative damages in all the macromolecules, including DNA [2–13];

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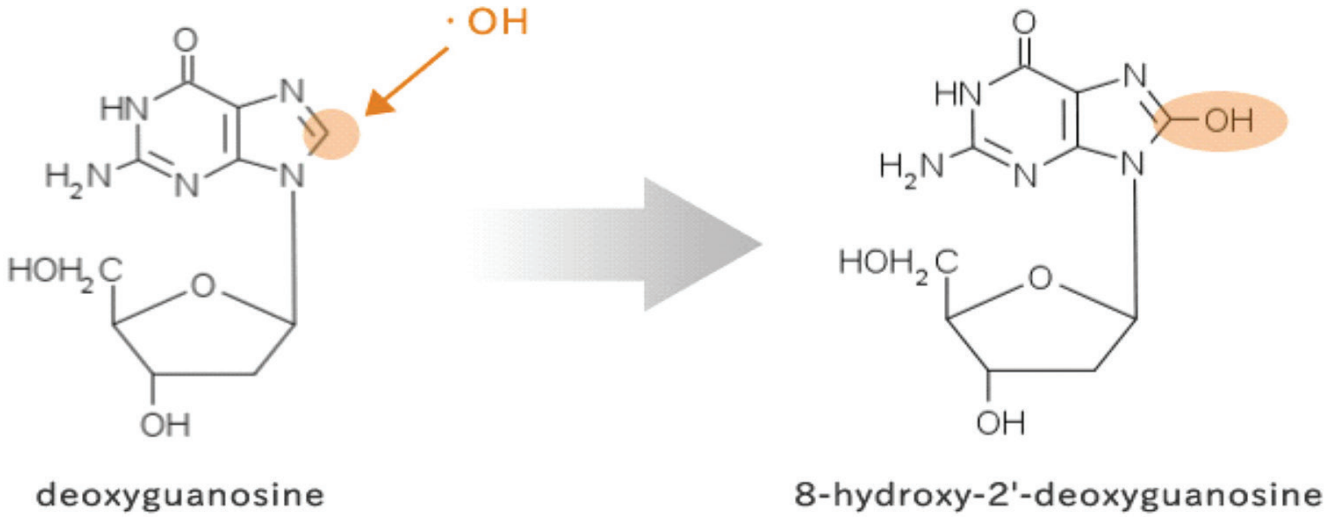

Figure 1. The ROS attack on the carbon in position eight of Gua oxidizes it into 8-OHGua and the non-oxidized nucleoside 2-dG into 8-OHdG.

for nucleic acids, the oxidative attack can directly occur on nucleotides whether assembled into the chain (*in situ*) or on the free triphosphoric deoxy-ribonucleotides (dNTP) of cytosolic or mitochondrial pools and subsequent mis-incorporation of their oxidized forms [14].

These alterations are physiologically counteracted by mechanisms that recognize the modified nucleobases, remove them from the chain, and synthesize new segments of DNA in conditions of balanced supply of non-oxidized nucleotides [15–17].

More than 20 oxidative modifications on nucleobases have been described [14]: those on guanine (Gua) were the most studied because its great ease of oxidation can earlier highlight the redox imbalance. The hydroxylated nucleoside 8-hydroxy-2-deoxy-guanosine (8-OHdG) (Fig. 1) has been seen elevated in many pathologies and it was widely considered one of the most sensitive markers of oxidative damage on DNA [14,18,19]. This damage is actively counteracted by two main pathways: base excision repair (BER) system that recognizes and removes every single altered nucleobase, and nucleotide excision repair (NER) system, by which entire sequences of damaged nucleotides are excised from the chains.

The relevance of both BER and NER come from the close implications of nucleobases oxidation on DNA replication and transcription. Indeed, it was previously described that a Gua hydroxylation *in situ* may cause substitutions Gua → thymine, and a mis-incorporation of 8-hydroxy-Gua (8-OHGua) may cause substitutions adenine → cytosine [20].

Despite BER and NER mechanisms, the persistence of oxidative attacks can lead to mutations that, directly or indirectly, could start pathogenetic processes, including neurodegenerative diseases [21–26]. Both Alzheimer's and PD are characterized by high oxidative imbalances, in which the same neurotransmitters are probably involved [27,28]; for them, it has been repeatedly hypothesized the pathogenetic implications of ROS levels, oxidative damage on DNA, and decrease in efficiency of BER and NER repair systems [29–32]. Particularly, it has been demonstrated that BER and NER weakening would result in a maintenance and progressive accumulation of mutations, especially in tissues, in which metabolic activity and cell replication persist even in late ages [33–42].

Our study focused on the measurement of 8-OHGua, 2-deoxy-guanosine (2-dG), and 8-OHdG as markers involved in the repair pathway of oxidative damage on Gua.

Until now, to 8-OHGua and 8-OHdG have often been attributed a generic role of oxidative markers; recalling the metabolic pathways in which these markers are involved, we have tried to take a step forward, attributing new and more specific significance to them.

The measurements of these markers were performed in urine samples collected from a large population of PD subjects ranked in H–Y stages. By statistical analysis, we have studied the relations between the urinary levels of 8-OHGua, 2-dG, and 8-OHdG and the clinical progression of pathology. A small population of subjects suffering from other parkinsonisms has also been studied initially to

highlight the differences between their oxidative framework and that which characterizes PD.

Materials and Methods

Samples collection

The second morning urines were collected from 198 subjects with idiopathic PD. Their age was of 72 ± 9 years [mean ± standard deviation (SD)] and the H–Y stages ranged from 0 to 4. The samples were collected even from 33 subjects with parkinsonisms, 20 females and 13 males of 73 ± 7 years.

Among the 84 PD subjects in H–Y stages from 0 to 2 (group H–Y < 2), 42 were females and 42 males; among the 114 subjects in H–Y stages 3 and 4 (group H–Y > 2), there were 64 females and 50 males. More in detail, two PD patients were in H–Y stage 0, 28 in stage 1, 54 in stage 2, 89 in stage 3, and 25 in stage 4.

After sampling, all urine samples were immediately frozen at −80°C until the analysis.

Hoehn–Yahr scales

The patient classification was performed by experienced neurologists using the Hoehn & Yahr scale (H–Y). The scale stages, ranged from 0 to 5, describes progressive worsening of clinical status: no signs of disease (stage 0), unilateral disease (stage 1), bilateral disease, without impairment of balance (stage 2), mild to moderate bilateral disease, some postural instability, physically independent (stage 3), severe disability, still able to walk or stand unassisted (stage 4), wheelchair bound or bedridden if not aided (stage 5).

Analytical methods

Creatinine (CR) and uric acid (UA) were analyzed with routine colorimetric (alkaline picrate) and enzymatic methods (uricase), respectively, while 8-OHdG and 2-dG were measured with high-performance liquid chromatography (HPLC) methods previously described [13]. 8-OHGua was assayed with a new chromatographic method summarized below.

After the alkalinization of samples by dilution of 100 µl of centrifuged urine with 900 µl Na_2HPO_4 100 mmol/l pH 9, a solid-phase extraction (SPE) was performed using 50 mg MF C18 Isolute cartridges (Biotage, Uppsala, Sweden), preconditioned with 500 µl acetonitrile, 1 ml water, and 1 ml Na_2HPO_4 100 mmol/l pH 9.

One hundred microliters of buffered urines, spiked with 8-OHGua (calibration standard solutions) and not spiked (unknown samples), were applied to SPE cartridges and eluted without vacuum. After a washing step with 100 µl Na_2HPO_4 100 mmol/l pH 9, the samples were finally eluted with 200 µl of a water: acetonitrile solution 80:20, (v/v), and 10 µl were injected into the chromatograph.

The HPLC system consisted of a 307 pump model and a 234 autosampler model with a 20 µl loop, both from Gilson (Villiers-le-Bel, France). The separation was performed on a 150 × 3.9 mm phenyl column InfinityLab Poroshell 120 PFP, 2.7 µm (Agilent, Santa Clara, CA).

The mobile phase was a NaH_2PO_4 20 mmol/l buffer solution (pH 5) flushed with a flow rate of 1.0 ml/minute: the pressure was about 210 kg/cm^2 and the capacity factor (k') for 8-OHGua was 2.4.

The detection was performed with an electrochemical "coulometric" detector (Coulochem II, ESA, Bedford, MA) fitted with a model 5011 high-sensitivity cell. The applied potentials at the first and second electrode were 0.00 and +0.45 volts, respectively. The current response was recorded at the second electrode only with a 2s time constant; the background current was about 40 nA with a full-scale deflection of 0.5 microA. Typical chromatograms are reported in Figure 2.

Statistical analysis

All markers, divided by pathology, stage, and gender were compared with analysis of variance test with a significant level fixed at 5%. Although the existence of many factors that change concurrently with the progress of the disease, as medication dosage, non-motor symptoms, etc., it would require a multivariate analysis, this was not be done due to the relatively small sample size for each group of patients.

Results

The new HPLC-electrochemical detection assay for 8-OHGua allowed an extraction recovery almost complete (99.2%). Thanks to the SPE selectivity based on both non-polar and secondary silanol interactions, the resolution of 8-OHGua peak from interferents in chromatograms was good.

Figure 2 shows some typical runs obtained under the above-described conditions. The precision was calculated by analysis of spiked samples (n = 8) in triplicate in three subsequent analyses: the intra- and inter-assay precision calculated were, respectively, 2.2% and 3.6%. The limit of detection, expressed as the double of the signal to noise ratio (S/N = 2), was 10 ng/l, and the analytical dynamic range was broad, between 0.5 and 2,000 µg/l.

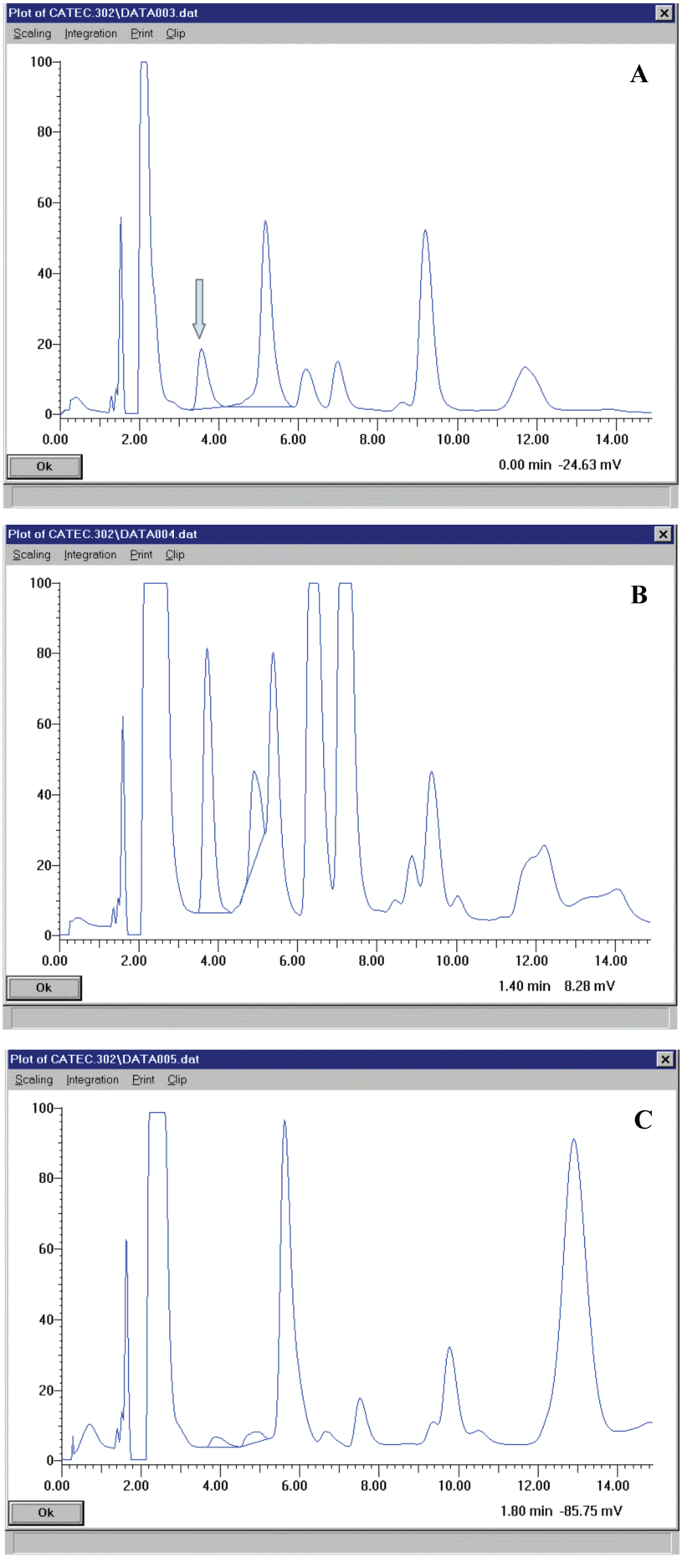

Figure 2. Typical chromatograms for a sample spiked with 8-OHGua to obtain a final concentration of 500 microg/L (A), a high level (B), and a low level (C) urine.

Table 1. Mean and SDs of urinary concentrations for the considered variables in the different H–Y stages of PD ranging from 0 to 4.

	8-OHdG/CR (ng/mg)		8-OHdG/UA (ng/mg)		2-dG/CR (ng/mg)		2-dG/UA (ng/mg)		8-OHdG/2-dG (pg/ng)		UA/CR (mg/g)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
H–Y 0 (*n* = 2)	187	40	707	223	310	300	1,214	1,237	1,015	851	270	28
H–Y 1 (*n* = 28)	380	499	1,608	1,385	312	255	885	540	2,239	2,467	314	214
H–Y 2 (*n* = 54)	264	412	870	1,220	3,978	10,505	21,183	60,080	1,429	2,325	285	112
H–Y 3 (*n* = 89)	523	1,834	1,583	4,797	1,734	4,337	5,186	12,118	2,641	5,301	330	131
H–Y 4 (*n* = 25)	464	1,002	2,032	4,377	3,003	7,236	9,686	20,255	2,410	3,624	340	160

Table 2. Mean and SDs of urinary concentrations for the considered variables measured below and above H–Y stage 2; each group it is also reported divided by gender.

	8-OHdG/CR (ng/mg)		8-OHdG/UA (ng/mg)		2-dG/CR (ng/mg)		2-dG/UA (ng/mg)		8-OHdG/2-dG (pg/ng)		UA/CR (mg/g)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
H–Y < 2 all subjects (*n* = 84)	270	400	927	1,194	3,422	9,742	18,127	55,674	1,478	2,243	287	117
H–Y < 2 females(*n* = 42)	247	498	673	1,195	6,962	15,567	24,914	55,187	413	512	350	130
H–Y < 2 males (*n* = 42)	282	355	1,054	1,201	1,652	4,401	14,734	56,893	2,010	2,579	250	100
H–Y > 2 all subjects (*n* = 114)	507	1,683	1,665	4,677	1,985	5,046	6,081	14,158	2,572	4,965	333	136
H–Y > 2 females (*n* = 64)	557	1,981	1,513	4,753	2,838	6,438	7,709	17,018	1,868	3,034	380	140
H–Y > 2 males (*n* = 50)	1,269	1,007	3,133	2,291	4,638	2,545	12,364	6,583	2,451	825	260	170

Table 1 shows the descriptive statistics for 8-OHdG, 2-dG, and UA in the urine of PD patients, divided by stage of H–Y scale. The classes HY 2 and 3 were much more represented than other because the enrolled subjects were a real sample of our medical center to which the patients are addressed when the neurological disease has already been discovered and in quite advanced stages.

Despite the sample sizes and variability of the data, it was already evident that the average values of 8-OHdG tend to increase with the progression of disease, both as CR (8-OHdG/CR) and as UA (8-OHdG/UA) ratio, reaching in stage 4 average values, even two to three times higher than the initial stages.

Instead, 2-dG, from the lowest values in stages 0 and 1 considerably increases in stage 2 and then it halves in stages 3 and 4, while UA progressively increases from stages 0 to 4.

To overcome the problems of sample size and obtain more comparable groups, PD subjects were ranked in two classes only: one that included the initial stages from 0 to 2 (n = 84 subjects, HY < 2) and another one with the advanced ones (stages 3 and 4, n = 114 subjects, HY > 2). In this way, both 8-OHdG/CR and 8-OHdG/UA increments from H–Y < 2 to H–Y > 2 became highly significant ($p < 0.001$, Table 2), as well as 2-dG/CR and 2-dG/UA decrements, while the changes in the UA/CR ratio were not statistically different ($p = 0.321$).

Moreover, the increases in 8-OHGua/CR and the decreases in 8-OHdG/8-OHGua ratios were not statistically different between H–Y < 2 and H–Y > 2 ($p = 0.401$ and $p = 0.109$, respectively, Table 3).

Subsequently, dividing each groups into genders, in HY < 2 class, the differences between males and females were significant, both for 2-dG/CR and for the 8-OHdG/2-dG ratio ($p < 0.001$): not significant, conversely, were those for 8-OHdG/CR ($p = 0.183$), 8-OHdG/UA ($p = 0.963$), 2-dG/UA ($p = 0.964$), and UA/CR ($p = 0.249$).

Table 3. Urinary levels of 8-OHGua in early and in advanced stages of PD as ratios of CR and 8-OHdG.

	8-OHGua/CR (mg/g)		8-OHdG/8-OHGua (mg/mg)	
	Mean	SD	Mean	SD
H–Y < 2 all subjects (*n* = 44)	113	183	10.3	12.1
H–Y > 2 all subjects (*n* = 40)	248	227	4.8	8.1

Table 4. Mean and SDs of the concentrations of Gua-related markers measured in PD and in parkinsonisms urine samples.

	8-OHdG/CR (ng/mg)		8-OHdG/UA (ng/mg)		2-dG/CR (ng/mg)		2-dG/UA (ng/mg)		8-OHdG/2-dG (pg/ng)		UA/CR (mg/g)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PD (n = 198)	453	1,492	1,496	4,152	2,314	6,418	8,842	29,557	2,321	4,506	320	130
Parkinsonisms (n = 33)	2,036	9,634	7,021	33,133	1,774	5,072	10,685	37,498	1,658	3,009	350	150

Instead, in HY > 2 class, besides 2-dG/CR and 8-OHdG/2-dG, also the differences for 8-OHdG/CR and 2-dG/UA were significant between males and females ($p < 0.001$ for all markers), while the changes for 8-OHdG/UA ($p = 0.844$) and UA/CR ($p = 0.170$) remained not significant.

For purposes of only observation, a statistical analysis was even performed to compare PD and parkinsonisms (Table 4). Significant differences were found for 8-OHdG/CR, 8-OHdG/UA ($p < 0.001$), 2-dG/UA ($p = 0.039$), and 8-OHdG/2-dG ($p = 0.024$), while the differences for 2-dG/CR ($p = 0.229$) and UA/CR ($p = 0.343$) were not significant.

Discussion

It was reported that the levels of 8-OHdG in extracellular fluids show a close link between ROS concentration and DNA alterations, and therefore it could be considered as a marker of "amount" of oxidation [14,18,19]. While the presence of free nucleoside 8-OHdG in urine has a not yet demonstrated origin [43,44], 8-OHGua, directly reflecting the action of BER pathway, maybe more specifically considered a marker of "efficacy" of DNA repair [45–47].

To increase the significance of 8-OHdG, not knowing in what way its excretion was related to the velocity of DNA remodeling, it has been proposed to express the concentration as the ratio of the corresponding non-oxidized 2-dG nucleoside. Therefore, it was possible to "normalize" the measurement of 8-OHdG as a function of the total amount of nucleoside [13,48–50].

However, 2-dG may also have another meaning that comes from the pathways that counteract the oxidative damage on DNA. There are two anabolic mechanisms for the synthesis of dNTPs, the *de novo* and the recovery or *salvage* pathways (Fig. 3); metabolic defects in both these ways are closely related to pathological conditions [51,52]. *De novo* is the quantitatively most important way for the synthesis of nuclear (nDNA) and mitochondrial DNA (mtDNA); it is a multi-stage process that starts from molecular precursors [53,54–56]. The *salvage* pathway, instead, starts directly from deoxynucleosides (dNs) coming both from DNA degradation and from diet. The dN enters into the cell by diffusion, mediated by specific permeases: subsequently, a first irreversible phosphorylation step converts dN into dNMP, which now becomes no longer permeable to the membrane. The dNMPs, via nucleoside monophosphate- and nucleoside diphosphate-kinases, are subsequently reversibly phosphorylated to dNDP and dNTP [57].

The key reaction of the *salvage* pathway is the irreversible phosphorylation of the dN to dNMP, catalyzed by kinases which undergo a negative feedback regulation from their substrates.

Another regulation system of the *salvage* pathway is that of nucleotidases that catalyze the "futile cycle" of dephosphorylation of dNMP to dN (Fig. 4) [58]. The relative activities of kinases and nucleotidases can thus establish the "verse" of the cycle dN → dNTP, and determine every time which direction prevails, anabolic or catabolic, according to the cellular needs.

When the DNA synthesis is not active, the excess of dNTPs inhibits the kinases, activates nucleotidases, and shifts the cycle in catabolic direction. Consequently, in the extracellular fluids, the dN concentration increases and also UA as a final catabolic product of purines. On contrary, when the endocellular dNTP pool is reduced, due to a reactivation of DNA synthesis or a decrease in the *de novo* pathway, the kinases phosphorylates dN, and calls other nucleosides from extra-cellular fluids with a consequent decrease in these of dN and UA [59].

Due to this close relationship between the inside/outside membrane balance for nucleosides and the nucleotides synthesis pathway, it is possible to propose new significances for 2-dG measurement in extracellular fluids, according to two different oxidative imbalance models named eustress and distress.

Oxidative eustress is an advantageous physiological condition in which a sudden increase in ROS levels, like for example, during an intense physical training, is counteracted by the antioxidant barrier, which progressively becomes stronger when the

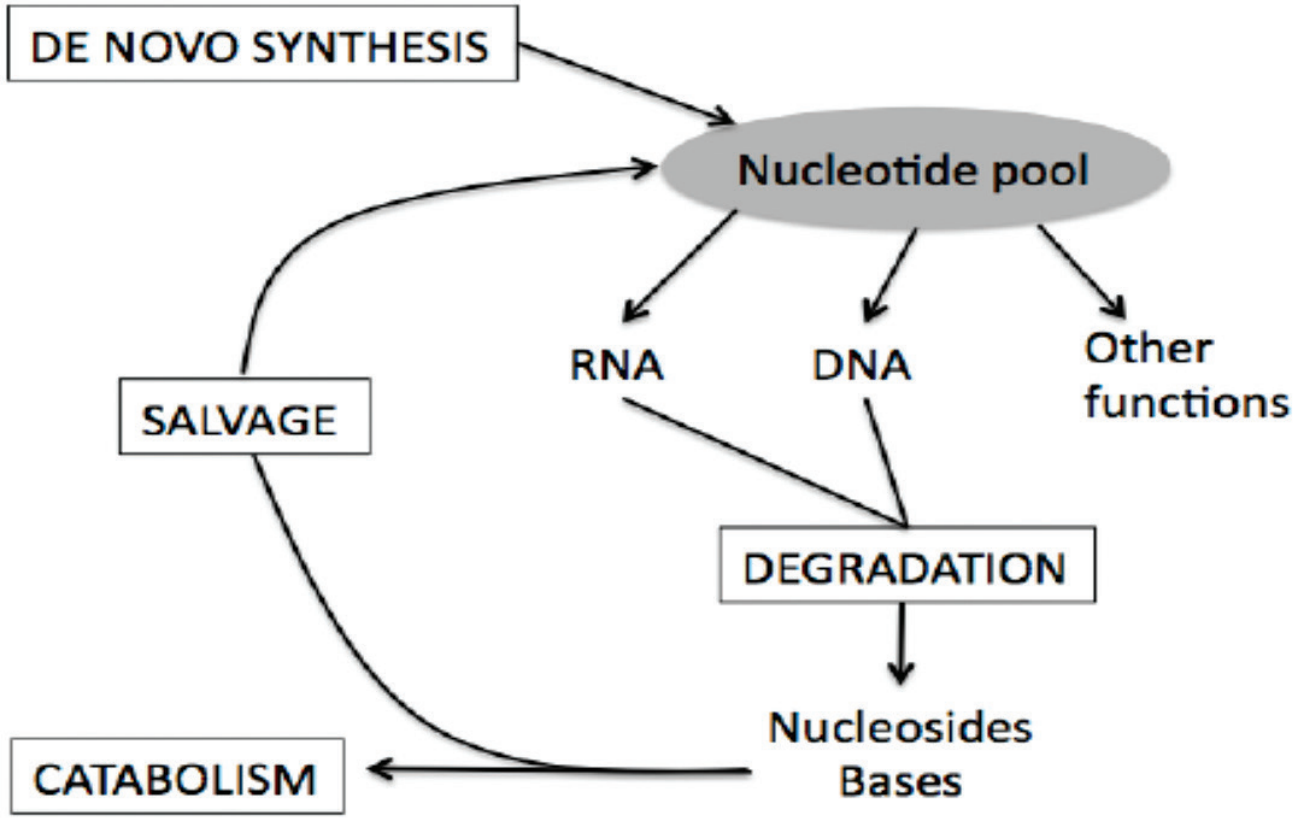

Figure 3. Scheme of nucleotide metabolism.

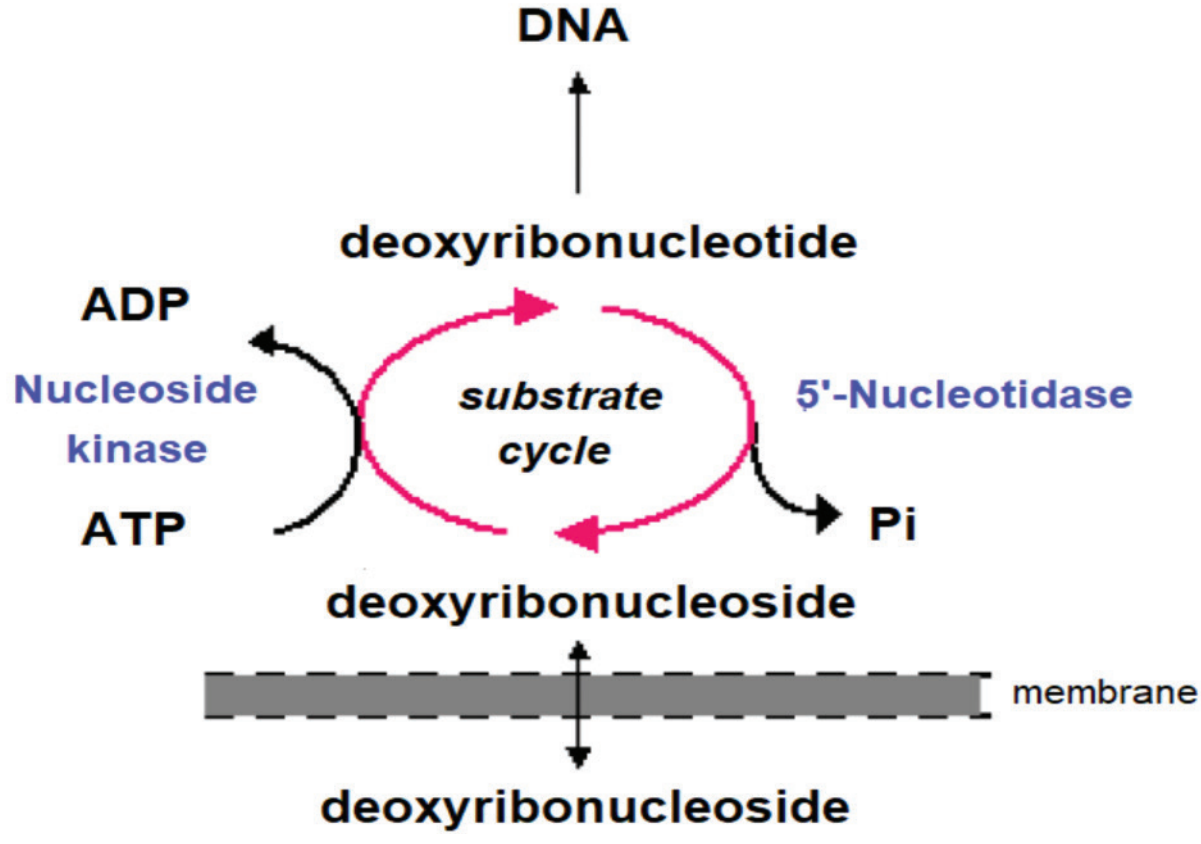

Figure 4. Schematic representation of the membrane equilibrium of deoxyribonucleosides and of their anabolic and catabolic cycles.

Table 5. Increase (↑) and decrease (↓) in Gua-related oxidative markers and their biochemical and patophysiological possible causes.

	Concentration in urine	Causes	
		Biochemical	Patophysiological
8-OHGua	↑	↑ BER action	Eustress or distress
8-OHdG/creat	↑	↑ 8-OHdG	Eustress or distress
		↓ CR	Renal insufficiency and other pathologies or conditions
2-dG/creat	↑	↑ 2-dG	Eustress
		↓ CR	Renal insufficiency and other pathologies or conditions
8-OHdG/UA	↑	↑ 8-OHdG	Eustress or distress
		↓ UA	Distress
2-dG/UA	↑	↑ 2-dG	Eustress
		↓ UA	Distress
8-OHdG/2-dG	↑	↑ 8-OHdG	Eustress or distress
		↓ 2-dG	Distress
8-OHGua	↓	↓ BER action	Healthy or reduced DNA turnover
8-OHdG/creat	↓	↓ 8-OHdG	Healthy or reduced DNA turnover
		↑ CR	Dehydration and other pathologies or conditions
2-dG/creat	↓	↓ 2-dG	Eustress/distress
		↑ CR	Dehydration and other pathologies or conditions
8-OHdG/UA	↓	↓ 8-OHdG	Healthy or reduced DNA turnover
		↑ UA	Eustress or renal insufficiency and other pathologies
2-dG/UA	↓	↓ 2-dG	Eustress/distress or renal insufficiency and other pathologies
		↑ UA	Eustress or renal insufficiency and other pathologies
8-OHdG/2-dG	↓	↓ 8-OHdG	Healthy or reduced DNA turnover
		↑ 2-dG	Eustress

oxidative attack repeats itself. Under eustress conditions, episodic oxidative damage may occur on DNA to which the cell responds recalling dN from extracellular spaces. In this case, the decreases in 2-dG and UA in urine can be considered as an effective response to the transient oxidative DNA damage.

Otherwise it happens in distress, a condition in which the oxidative imbalance, typically linked to pathological conditions such PD, becomes chronic and the oxidative insult is not episodic but constant and quantitatively progressive. In this way, dN is constantly subtracted from the membrane equilibrium to supply the *salvage* anabolic pathway of nucleotides.

Since in chronic oxidative imbalance, the physiological level of endocellular dNTP pool is never reached, the concentrations of 2-dG and UA in extracellular fluids are constantly low.

On these considerations, it was so possible to propose some interpretative hypotheses concerning the measurements of 8-OHGua, 8-OHdG, 2-dG, and UA in PD urine (Table 5).

First, the levels of 8-OHdG/CR and 8-OHdG/UA are already high in the early stages H–Y < 2 (Table 2), when compared with data previously reported in a not ill elderly population (51 ± 49 ng/mg and 199 ± 216 ng/mg, 8-OHdG/CR and 8-OHdG/UA, respectively) [11].

As a demonstration of the persistent and increasing oxidative attack in PD, the values found in early stages tend to increase further on, until doubling in the H–Y > 2 group.

The 2-dG levels in H–Y < 2, on the other hand, were comparable with those of a control population only for 2-dG/CR ratio (2,805 ± 3,914 ng/mg). Nevertheless, they were much higher if expressed as UA ratio (6,671 ± 8,201 ng/mg) [11]. It is also to be observed that, in the stages H–Y 0 and 1, both ratios 2-dG were much lower than the control group and that the strong increase concerns the stage 2 only (Table 1).

Looking at the progressive constant rise of the 8-OHdG, it is to think that the response to the oxidative damage on DNA in PD is weak and insufficient. In fact, during disease, no strong decreases were

observed in extracellular 2-dG levels that would have indicated activation of *de novo* and *salvage* pathways. Apparently, this phenomenon occurs in early stages but then it stops or slows down in advanced stages.

Moreover, in addition to a not-decrease in 2-dG/CR ratio, there is also an initial increase in the 2-dG/UA ratio, which is most likely due to a relative decrease in UA concentrations and, therefore, to a slowing of purine catabolism.

Although the variations of UA concentration were not statistically significant between the H–Y stages, this data reinforces the following hypothesis. At the beginning of the disease, while determining a progressive oxidative damage on DNA, the ways of nucleosides recovery, synthesis of dNTP, damage repair, and catabolism of oxidized nucleobases would appear blocked or strongly slowed down.

In the later stages HY > 2, together with a noticeable increase in both 8-OHdG/CR and 8-OHdG/UA ratios, 2-dG tends to decrease both in relation to CR and to UA. This fact, unfortunately late, would be considered positive because it is significant of a weak reactivation of DNA turnover.

Furthermore, between H–Y < 2 and H–Y > 2 the 8-OHGua/CR doubles in urine, albeit without statistical significance, which would indicate that the BER activity would be present in advanced stages, despite the persistence of oxidative attack. Particularly, the mean decreases in the 8-OHdG/8-OHGua ratio, also not statistically significant, would suggest that, at least in some subjects, the BER action would be successful. In fact, looking at the increase in both 8-OHdG and 8-OHGua between HY < 2 and HY > 2, the decrease in 8-OHdG/8-OHGua ratio would indicate that the 8-OHGua (denominator) increases more than the 8-OHdG (numerator) and therefore that the removal mechanism of oxidized nucleobase would successfully counteract the oxidative attack.

Since in our study, the different therapeutic strategies used for each subject have been not considered, further investigations become necessary in order to understand if and how the treatments (therapeutic cocktail, physical activity, and functional foods) can counteract oxidative damage strengthening the physiological mechanisms of repair.

Interesting is also the gender-related differences in markers concentration between PD stages. It has been previously shown that the higher disease prevalence, the earlier onset of PD dementia, and the greater severity of cognitive decline were prevalent in males than females [60].

In our study, also DNA oxidative markers in H–Y > 2 appear higher in males than in females. Indeed in males, the almost double values of 8-OHdG and 2-dG would show a greater oxidative attack than in females and a lower damage response.

This gender difference was not equally evident in the early stages in which the values of 8-OHdG were not different between the sexes. Rather, it was observed that the lower levels of 2-dG in males seemed to suggest in them a more effective response to oxidative damage.

Eventually, the comparison between PD and parkinsonism has opened a scenario that necessarily requires further studies (Table 4). It is well known that parkinsonisms, albeit with similar symptoms, differs from PD. The symptoms are almost never characterized by progressive neurodegeneration. Therefore, it is strange to observe the levels of 8-OHdG, and so the amounts of oxidative damage, much higher in parkinsonisms than in PD. Certainly, the etiological and clinical heterogeneity of parkinsonisms, the gender- and stage-related differences in PD and the large numerical difference between groups are so important factors that more detailed studies are needed.

Conclusions

Our data seem to show that, in PD, the response to redox imbalance, considered as removal and replacement of the oxidized nucleotides on DNA is slow and insufficient to counteract effectively the oxidative insult.

Indeed, as the disease gets worse, urinary 8-OHGua increases and 2-dG decreases, showing a removal of the oxidized nucleobase from DNA chains and a recovery of a native nucleoside for its replacement. However, at the same time, the 8-OHdG concentrations increase more quickly with the HY stage, suggesting that the oxidative damage becomes chronic, progressively worsens, and is not sufficiently counteracted from repair mechanisms.

If referred to gender, the progression of oxidative damage is more marked in males than in females and correlates, in a suggestive way, with the already highlighted gender-related prevalence of pathology.

In conclusion, while in PD there seems to be a correlation, perhaps a causal link, between oxidative damage and the progression of neuronal

damage, it is surprising that the parkinsonisms do not have neurodegeneration, even with an oxidative insult higher than PD.

Furthermore, studies will be, therefore, necessary to confirm these data in larger and more homogeneous populations.

Declaration of Interest

The authors declare that they have no conflict of interest with the topics discussed and that the study was completely self-financed without intervention of pharmaceutical companies or any other sponsor. This manuscript has been read and approved by all the authors; each author declares that the manuscript represents an original work never previously published either in whole or in part.

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