Taylor & Francis
Taylor & Francis Group

Scandinavian Journal of Clinical and Laboratory Investigation

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iclb20

An *in silico* kinetic model of 8-oxo-7,8-dihydro-2-deoxyguanosine and 8-oxo-7,8-dihydroguanosine metabolism from intracellular formation to urinary excretion

Anders Jorgensen, Maria Bremholm Thygesen, Uffe Kristiansen & Henrik Enghusen Poulsen

To cite this article: Anders Jorgensen, Maria Bremholm Thygesen, Uffe Kristiansen & Henrik Enghusen Poulsen (2021): An *in silico* kinetic model of 8-oxo-7,8-dihydro-2-deoxyguanosine and 8-oxo-7,8-dihydroguanosine metabolism from intracellular formation to urinary excretion, Scandinavian Journal of Clinical and Laboratory Investigation, DOI: 10.1080/00365513.2021.1969682

To link to this article: https://doi.org/10.1080/00365513.2021.1969682

SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION
https://doi.org/10.1080/00365513.2021.1969682

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

An *in silico* kinetic model of 8-oxo-7,8-dihydro-2-deoxyguanosine and 8-oxo-7,8-dihydroguanosine metabolism from intracellular formation to urinary excretion

Anders Jorgensen[a,b], Maria Bremholm Thygesen[c], Uffe Kristiansen[c] and Henrik Enghusen Poulsen[a,d]

[a]Institute of Clinical Medicine, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark; [b]Psychiatric Center Copenhagen (Rigshospitalet), Mental Health Services of the Capital Region, Copenhagen, Denmark; [c]Department of Drug Design and Pharmacology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark; [d]Department of Clinical Pharmacology, Copenhagen University Hospital Bispebjerg and Frederiksberg, Copenhagen, Denmark

ABSTRACT

Oxidatively generated DNA damage is of paramount importance in a wide range of physiological and pathophysiological processes. Urinary 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG) is often used as an outcome marker in studies on the role of oxidatively generated DNA damage, but its exact relation to intracellular damage levels and variations in DNA repair have been unclear. Using a new approach of quantitative kinetic modeling inspired by pharmacokinetics, we find evidence that in steady state – i.e. when systemic consequences of given change in damage or cellular removal rates have stabilized - the urinary excretion of 8-oxodG is closely correlated to rates of damage and intracellular 8-oxodG levels, but independent of the rate of cellular removal. Steady state was calculated to occur within approximately 12 h. A similar pattern was observed in a model of the corresponding RNA marker 8-oxo-7,8-dihydroguanosine (8-oxoGuo), but with steady-state occurring slower (up to 5 d). These data have significant implications for the planning of studies and interpretation of data involving urinary 8-oxodG/8-oxoGuo excretion as outcome.

HIGHLIGHTS

- The kinetics of 8-oxodG/8-oxoGuo formation, removal and excretion were simulated *in silico*.
- The model was based on existing data on 8-oxodG/8-oxoGuo levels and removal/excretion rates.
- Intracellular 8-oxodG/8-oxoGuo was closely correlated with urinary excretion in steady state.
- Changes in removal rates did not influence urinary excretion of 8-oxodG/8-oxoGuo.

ARTICLE HISTORY
Received 11 February 2021
Revised 1 July 2021
Accepted 26 July 2021

KEYWORDS
DNA; RNA; oxidative stress; DNA repair; 8-oxo-7,8-dihydro-2-deoxyguanosine; 8-oxo-7,8-dihydroguanosine; kinetics

Introduction

Urinary 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG) is the most well-validated marker of systemic oxidative stress on DNA; a phenomenon of paramount importance in many physiological and pathophysiological processes, such as aging, cancer development and neurodegeneration. The compound is formed by the oxidatively generated modification of guanine nucleotides by reactive oxygen species (ROS). It is found in isolated DNA, plasma, urine, and other matrices and the corresponding ribonucleoside, 8-oxo-7,8-dihydroguanosine (8-oxoGuo), is likewise found in isolated RNA, plasma (unpublished), urine and other matrices [1–3]. The exact intracellular origins of 8-oxodG (and 8-oxoGuo) and the processes leading to their eventual excretion in urine have not been established, and the enzymatic repair processes initially assumed to remove the oxidized guanine moiety (e.g. base excision repair) are apparently not responsible [4]. Oxidatively generated damage to DNA by 2-nitropropane in experimental animals induces 8-oxodG in several organs, and the number of intra-DNA 8-oxodG units generated roughly corresponds to the number of 8-oxodG molecules found in urine the following 0–24 h, where the levels of intra-DNA lesions in the organs are back to baseline level [5]. In urine, both the oxidized base and nucleoside are found; the oxidized base in about five-fold higher concentrations [6]. Our group has focused on the oxidized nucleosides, because this allows differentiation between the DNA and the RNA form. We have demonstrated this to be clinically important, particularly in type-2 diabetes where the ribonucleoside, but not the 2′-deoxyribonucleoside, is prognostic for the all-cause mortality and mortality from cardiovascular disease [7]. We have also found that neuropsychiatric disorders and interventions may have different impact on the DNA *vs.* the RNA marker [8,9].

When conducting studies with 8-oxodG as an outcome, sometimes one is faced with the argument that a change in urinary 8-oxodG excretion could both be caused by altered oxidative stress on DNA or altered DNA repair. For example, if an increase in urinary excretion is observed after a given intervention or in comparison of two groups, this could be

CONTACT Anders Jorgensen anders.01.joergensen@regionh.dk Psychiatric Centre Copenhagen (Rigshospitalet), Mental Health Services of the Capital Region, Edel Sauntes Allé 10, Copenhagen DK-2100, Denmark

Supplemental data for this article can be accessed online at https://doi.org/10.1080/00365513.2021.1969682

interpreted as increased oxidative stress on DNA (presumably a bad thing) or an increase in DNA repair (presumably a good thing). Clearly, these ambiguities affect the conclusions and biological interpretations that can be made from studies involving urinary 8-oxodG measurement as an outcome.

The purpose of this study was to build a detailed kinetic model of the full pathway of 8-oxodG turnover, from its formation within the cells to its urinary excretion, based on all the available evidence on concentrations in various biological compartments as well as cellular removal and excretion rates. Using this model, we wished to investigate how theoretical changes in cellular 8-oxodG formation and removal would affect a) concentrations/excretion rates in relevant biological compartments, such as DNA, plasma and urine, and b) the temporal relationship between a change in cellular formation or removal and relaxation to a new steady state in these compartments.

Methods

We built a quantitative kinetic model based on existing data on the formation, removal, distribution and excretion of 8-oxodG. The model was based on the following data sources and assumptions:

1. The model consisted of three overall compartments (Figure 1):
   a. *An intracellular compartment*, where 8-oxodG is formed in DNA [10], removed by a process which has not been clarified, but most likely involves some form of enzymatic excision, and released to the cytosol. Formation of 8-oxodG also occurs in the nucleotide pool [4], as well as enzymatic purine 'salvage' and DNA reincorporation of 8-oxodG [11], but quantitative data on these processes are not available, and the term 'DNA' is used with these caveats in mind. Likewise, and because of the standing discussion on which repair process are responsible for removal of oxidized guanine moieties from DNA, we use the broad term 'cellular removal', that indicates removal from the cell by unknown processes from DNA or the nucleotide pool. Hence, for the purpose of this study, the intracellular compartment is considered a 'black box' of formation and removal of 8-oxodG (and 8-oxoGuo); i.e. the model only assumes that the oxidation products are formed in the cells and that this eventually leads to their excretion into urine. This assumption is valid as judged by our previous experimental study [5].
2. *Extracellular compartments* comprising plasma and other tissues of distribution [2,3].
3. *Urine*, to which circulating 8-oxodG is excreted in full [12–14].

Absolute numbers of 8-oxodG molecules at a given time point were used for each compartment. Using the guanine fraction of nucleobases; the amount of nucleobases per cell (www.genome.gov), and estimates of the total number of cells in the human organism [15], the absolute number of organismal undamaged dG in DNA were calculated as:

$$0.25\ (\text{guanine fraction of nucleobases}) \cdot 6 \cdot 10^{9}\ \text{nucleobases in DNA per cell} \cdot 3.72 \cdot 10^{13}\ \text{cells per organism} = 5.6 \cdot 10^{22}\ \text{dG/organism}.$$

Please note that this represents the theoretical maximum of dG subject to oxidation.

The calculation of a removal rate constant (RRC) was based on the results from Dizdaroglu's group, who found a cellular RRC in human lymphoblasts of $0.0127 \pm 0.0012\ \text{min}^{-1}$ [16]. From this, the daily RRC was calculated as:

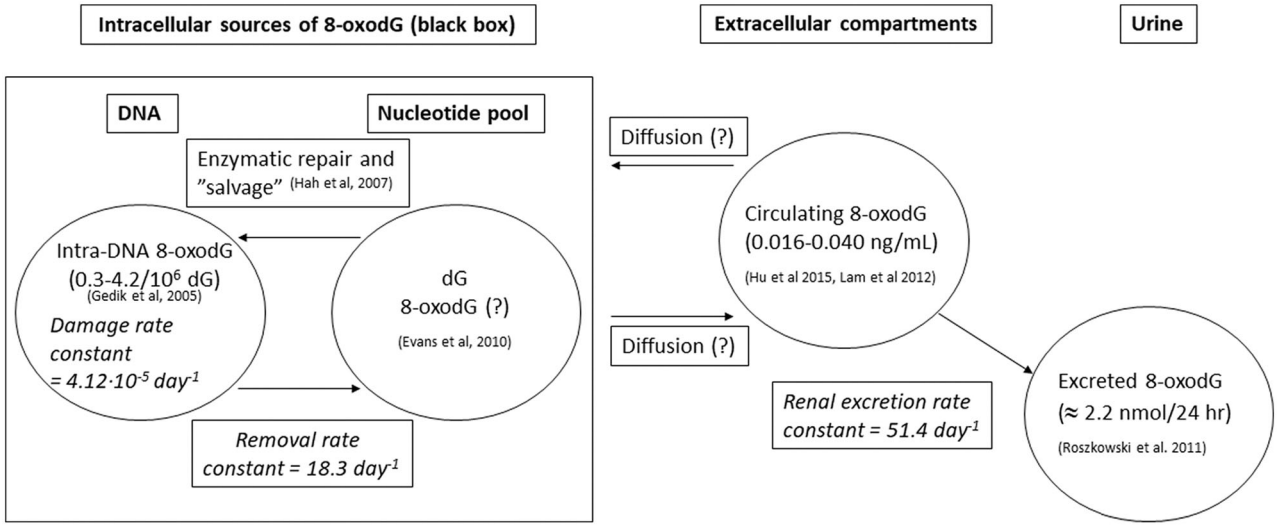

Figure 1. Graphical presentation of the overall structure underlying the proposed kinetic model of 8-oxodG metabolism from formation to urinary excretion. Known damage levels and 8-oxodG amounts are shown, including references. If quantitative data on a given factor is not known, this is shown by (?). Rate constants calculated for use in the model are shown in italic. See text for details.

$$RRC = 0.0127\ min^{-1} \cdot 60\ min/h \cdot 24\ h/d = 18.3\ d^{-1}.$$

Based on this constant, a damage rate constant (DRC) was calculated. We used the consensus levels of 8-oxodG per intact dG as found by the ESCODD network (0.3–4.2 8-oxodG/10^6 dG) [10]. For simplicity, we used the mean of this level (2.25 8-oxodG/10^6 dG) as a reasonable estimate. We assumed steady state for 8-oxodG in DNA, in which damage rate = cellular removal rate, i.e.:

$$\text{Total dG} \cdot \text{DRC} = \text{total 8-oxodG in DNA} \cdot \text{RRC} \Leftrightarrow$$
$$\text{DRC} = \text{total 8-oxodG in DNA} \cdot \text{RRC/Total dG} \Leftrightarrow$$
$$\text{DRC} = 2.25\ \text{8-oxodG}/10^6\ \text{dG} \cdot 5.6 \cdot 10^{22}\ \text{dG}) \cdot 18.3\ d^{-1}/5.6 \cdot 10^{22}\ \text{dG} = 4.12 \cdot 10^{-5}\ d^{-1}$$

By an alternative strategy, using the 'hit rate' of 10^5 8-oxodG produced per day per cell [17], a similar result was obtained (not shown). In the mathematical model, the above-mentioned levels of organismal undamaged dG were set to constant, corresponding to the physiological situation in which dG is not depleted by the damage and repair, but continously replenished for DNA synthesis.

Based on plasma elimination kinetics from a pig study [12], the extracellular distribution of 8-oxodG was found to be a two-compartment system (see Supplementary Material). Hence, the model consists of two transition compartments, a central compartment and a peripheral compartment. The volumes and distribution rate constants of these compartments were calculated using data from the study by Loft et al. Volume values were allometrically scaled to fit the 'Reference Man' [18], yielding a central compartment volume of 20 L and a peripheral compartment volume of 45 L. These compartments are mathematical constructs, and the biological nature of the matrices, or the exact way that intracellularly formed 8-oxodG would distribute in these compartments under physiological circumstances, cannot be determined from the data. However, the central compartment can be assumed to comprise plasma (3.5 L) and the interstitial space (around 10.5 L), and only data from this compartment is presented. In steady state, the addition of a peripheral distribution compartment does not influence the results.

Intravenously injected 8-oxodG is fully excreted into urine within 4 h [12], and 8-oxodG passes freely into the urine by glomerular filtration [19]. Therefore, renal clearance of 8-oxodG = glomerular filtration rate, which in young men is around 180 L/d. Hence, the 8-oxodG excretion rate constant (ERC) can be calculated from the plasma volume and the GFR:

$$ERC = 180\ L/d/3.5\ L = 51.4\ d^{-1}$$

We and others have recently implemented an analysis of the ribonucleotide analog of 8-oxodG, 8-oxoGuo. Because RNA is presumably degraded rather than repaired, the urinary excretion of 8-oxoGuo is expected to stem from this process [1]. Here, a tentative model of 8-oxoGuo kinetics was made. The model assumes that 8-oxoGuo is excreted to the urine in a similar rate as 8-oxodG. The model was based on the following estimates:

a. Damage levels of the ribonucleotide of five 8-oxoGuo per 10^6 Guo [20,21].

Most RNA species are degraded within 5–12 h [22]. Baseline degradation half-life was set to 8h.

Total RNA per cell =20 pg.

Guanine fraction RNA = 20 pg $\times 0.25 = 5$ pg = 0.01043 pmol = 0.01043×10^{-12} mol.

G (in RNA) per cell (n)=0.01043×10^{-12} mol$\times$ 6.022×10^{23} mol^{-1} =0.00628×10^{11}.

G (in RNA) per organism (n)= 0.00628×10^{11} $\times 3.72 \times 10^{13}$ cells per organism = $0.02336 \times 10^{24} = 2.336 \times 10^{22}$.

Degradation rate constant (based on half-life of 8 h) = ln(2)/0.333 d= 2.08 d^{-1}.

DRC = 5.0 8-oxoGuo/10^6 Guo$\cdot 2.336 \times 10^{22}$ Guo$\cdot$2.08 d^{-1}/ 2.336×10^{22} Guo = $1.04 \cdot 10^{-5}$ d^{-1}.

The kinetic modeling was made in the Berkeley Madonna software© version 10 (2020), Albany, CA, USA. A graphical presentation of the model is shown in Supplementary Figure 1. Integration method was Runge–Kutta 4 with dt = 0.001 ds. Upon request, the model is available for interested researchers.

Results

We did simulations on changes in damage and cellular removal/degradation rate constants for 8-oxodG/8-oxoGuo, respectively (Figure 2(A,B)). To mimic plausible physiological effects of a given intervention, we used 25% and 50% increase/decrease for each constant; however, the results of the model were stable at up to a 100-fold change. Figure 2(A), upper panel, shows that when changes in the DRC occur, both the DNA, central compartment ('plasma'), and urinary levels of 8-oxodG change within 12 h into a new steady state that closely correlates to the new level of intracellular damage. In contrast, when changes in the cellular RRC occur (Figure 2(A), lower panel), levels of DNA 8-oxodG change with inverse proportionality, whereas levels of central compartment and urinary 8-oxodG are unaltered from baseline levels except for a brief adjustment occurring immediately after the change and lasting around 12 h. These findings apply to both directions of change (increase or decrease, respectively). For the 8-oxoGuo model, a similar pattern is observed, but with steady-state occurring slower (up to 5 d) due to the longer degradation time (Figure 2(B)).

Discussion

To our knowledge, this is the first attempt to quantitatively model the entire 8-oxodG/8-oxoGuo turnover, from its intracellular formation to its eventual urinary excretion, based on the existing available data on background levels and cellular removal rates. We find that in steady state and within physiological fluctuations, urinary 8-oxodG/8-oxoGuo excretion per time unit is a direct reflection of intracellular formation of 8-oxodG/8-oxoGuo, whereas changes in cellular removal/degradation rates do not manifest themselves by changes in plasma levels or urinary excretion, except transiently between different steady states. The system adheres to first-order kinetics, in which the rate-limiting step is formation rather than removal, which does not reach saturation in any reasonable physiological situation. In other words, although the removal or degradation of

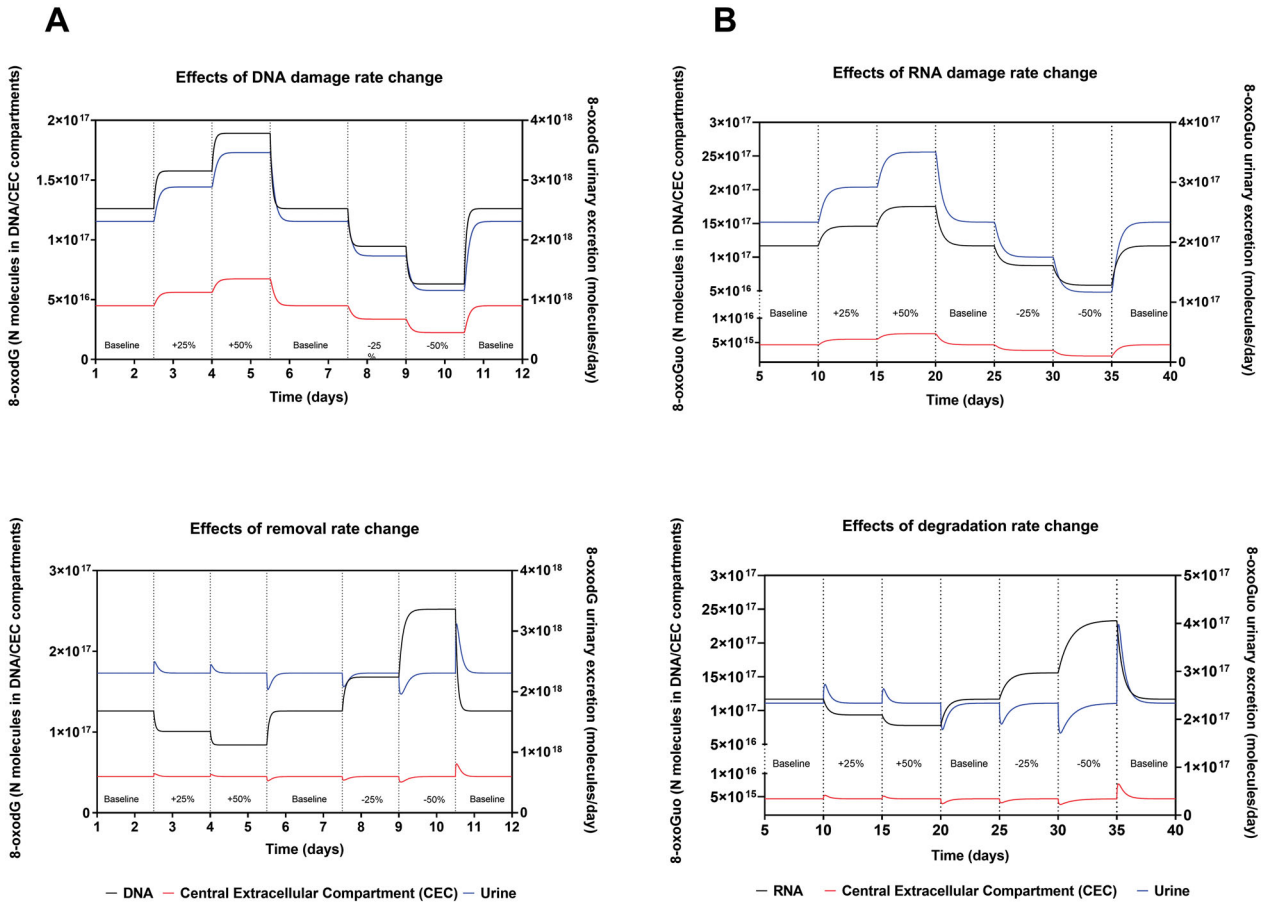

Figure 2. Kinetic modeling of total 8-oxodG (A) and 8-oxoGuo (B) molecule amounts on a given time point in DNA (A)/RNA (B) (black, left Y-axis), the central extracellular compartment (which can be assumed to include plasma) (red, left Y-axis), and 8-oxodG/8-oxoGuo renal excretion rate (molecules/day) (blue, right Y-axis) during theoretical changes in oxidative DNA/RNA damage rate constants (upper panel) and 8-oxodG/8-oxoGuo removal rate constants (lower panel). Note that with changes in damage, 8-oxodG/8-oxoGuo in plasma and urine fluctuates in almost complete correlation with DNA/RNA levels, whereas during changes in removal, 8-oxodG/8-oxoGuo in plasma and urine rapidly returns to baseline values after a brief adjustment, in spite of significantly changed amounts of 8-oxodG in DNA/RNA. Note that the time to steady state is longer for 8-oxoGuo than for 8-oxodG (up to 5 d depending on the degradation time). See text for details of the model.

oxidized guanine species from the cell relies on mechanisms that can perform this (e.g. repair or similar processes), the urinary excretion cannot be interpreted as a measure of these processes, but is a measure of the formation rate, which in turn corresponds to oxidative stress on nucleic acids. The markers found in urine cannot differentiate between oxidation in the nucleic acid or the nucleotide pools; however, when used as a biomarker this differentiation is not important. The half-life of induced intra-DNA 8-oxodG modifications in different cell types and species was recently summarized by Cadet et al. and found to be around 1–4 h after cellular insults, such as gamma irradiation [23]. Because steady state occurs after approximately five half-lives, these experimental data correspond well with our *in silico* study, in which steady-state occurred after around 12 h. Steady state occurred slower for 8-oxoGuo as a consequence of the longer degradation time. A recent study on 8-oxodG and 8-oxoGuo excretion 0–5 d after UV exposure in humans with different skin melanization [24] seem to support differences in 8-oxodG/8-oxoGuo kinetics that could be compatible with our results, i.e. a slower response of 8-oxoGuo as compared to 8-oxodG changes after an intervention. The study also indicated differences in the speed of repair within skin types, and our model incorporates such physiological variation, because it was run at ±50% changes in formation and removal, and was stable at up to a 100-fold change.

As noted in the methods section, it is important to emphasize that the exact intracellular process underlying urinary 8-oxodG excretion has not been identified. Oxoguanine glycosylase 1 (OGG1), which was initially considered to be the most likely source, excises only the oxidized nucleobase, which is correspondingly found to be reduced in urine of *Ogg1*$^{-/-}$ knockout mice [25]. Evans et al. recently found no difference in urinary 8-oxodG excretion in genetically modified mice with deficiencies within the nucleotide excision repair system [26]. However, although the intracellular mechanism underlying the urinary excretion of 8-oxodG is unknown, it is still considered that it most likely involves an enzymatic repair process [27]. There is experimental data to support that the Nudix hydrolase NUDT1, which sanitizes the nucleotide pool of 8-oxodG-triphophate (8-oxodGTP), is a candidate enzyme that could ultimately yield free 8-oxodG [28]. Overall, given the threat that damage to DNA or its building blocks constitute to cellular and organismal survival, it would seem

unlikely that there is no active (enzymatic) process underlying the release of extracellular 8-oxodG.

The removal rate used in our model was calculated from a general rate of DNA repair, and not the specific rate of the unknown process that results in the release of free 8-oxodG. However, in the study by Haghdoost et al., which suggested a role for NUDT1 in the extracellular release of 8-oxodG, effects of radiation on 8-oxodG release to the extracellular medium (with or without altered NUDT1 expression) could be detected after 1 h [28]. Importantly, our early experimental study showed that a challenge with 2-nitropropane in rats resulted in excess liver, kidney and bone marrow concentration of intra-DNA 8-oxodG, which was paralleled by an corresponding excess excretion of urinary 8-oxodG in the following 24 h and already detectable in the 0-6 h post-treatment samples [5]. Collectively, we consider it to be the most likely scenario that removal of 8-oxodG follows the same enzyme kinetics as the removal of other oxidatively generated DNA modifications, although subtle differences between different enzyme systems may clearly exist.

The findings of our study have implications for the planning of experiments involving urinary 8-oxodG (and/or 8-oxoGuo) as outcome measure. After a change in damage (e.g. from an intervention), the time to a new steady state should be taken into account when designing the study. Second, a theoretical intervention or condition that *only* affects aspects of DNA repair is unlikely to affect the urinary excretion of 8-oxodG more than transiently, even though intra-DNA levels of 8-oxodG will change. In the extreme case of no repair at all, excretion will obviously seize, accumulation will increase and levels incompatible with life will occur much before the natural lifespan. However, a general biological principle of enzymatic DNA (and nucleotide pool) repair has been observed to be the existence of functional redundancy (e.g. by back-up repair pathways), due to which a complete loss of effect of one specific enzyme (e.g. in genetically modified animals) does not completely abolish repair capability for the specific substrate of that enzyme [27,29].

Our model predicts the urinary flux of 8-oxodG to be around $2.25 \cdot 10^{18}$ molecules/d (Figure 2), which corresponds to 3.73 micromoles/d (see Supplementary Material for calculation). The 24 h 8-oxodG excretion measured in healthy humans has been found to be considerably lower (in the range of 2–20 nanomoles/d) [13,14]. As noted in the Methods section, we based our baseline levels of intra-DNA 8-oxodG on the full theoretical organismal amount of dG, and hence our model indicates that under biological circumstances, not all of the organismal dG is target for oxidation, e.g. because of protection by supercoiling or by protecting proteins.

With respect to the potential contribution of the nucleotide pool to urinary 8-oxodG excretion, evidence suggests that dGTP is asymmetrically present in the nucleotide pool as compared to dG in DNA, as it constitutes 5% or less in most cells. On the other hand, the nucleotide pool is more susceptible to ROS-induced damage [30]. Also, as mentioned previously, there is a proposed cycle of 8-oxodGTP formation and breakdown in the nucleotide pool, allowing for salvage and (re)incorporation of 8-oxodG into DNA [11]. Due to the uncertainty of the relative contribution of these factors, we did not include 8-oxodG formed in the nucleotide pool in the model, and thus we cannot rule out that the amount of 8-oxodG in the 'black box' may be somewhat larger. Finally, the model does not take into account that *in vivo*, the levels of 8-oxodG excretion in urine represent a weighted average of the compartmentalized formation and removal of 8-oxodG in different cells and tissues, which may differ substantially [1,31]. However, none of these caveats would influence the overall kinetics predicted by the model.

In conclusion, using a new approach of quantitative kinetic modeling, we find evidence that in steady state and within the physiological range, the urinary excretion of 8-oxodG is a reflection of overall systemic oxidative stress on DNA (and likely the nucleotide pool), because it is strongly correlated to intracellular 8-oxodG levels and independent of the cellular removal rate. Steady state can be expected to occur within 12 h after a given change. A similar pattern was observed for the 8-oxoGuo kinetics, albeit with a longer time to steady state. These data have significant implications for the planning of studies and interpretation of data involving urinary 8-oxodG/8-oxoGuo as outcome markers.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by Eva and Robert Voss Hansen Foundation and Psychiatric Center Copenhagen (AJ).

References

[1] Poulsen HE, Nadal LL, Broedbaek K, et al. Detection and interpretation of 8-oxodG and 8-oxoGua in urine, plasma and cerebrospinal fluid. Biochim Biophys Acta. 2014;1840(2):801–808.

[2] Lam PM, Mistry V, Marczylo TH, et al. Rapid measurement of 8-oxo-7,8-dihydro-2'-deoxyguanosine in human biological matrices using ultra-high-performance liquid chromatography-tandem mass spectrometry. Free Radic Biol Med. 2012;52(10):2057–2063.

[3] Hu CW, Cooke MS, Tsai YH, et al. 8-Oxo-7,8-dihydroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanosine concentrations in various human body fluids: implications for their measurement and interpretation. Arch Toxicol. 2015;89(2):201–210.

[4] Evans MD, Saparbaev M, Cooke MS. DNA repair and the origins of urinary oxidized 2'-deoxyribonucleosides. Mutagenesis. 2010;25(5):433–442.

[5] Deng XS, Tuo J, Poulsen HE, et al. Prevention of oxidative DNA damage in rats by brussels sprouts. Free Radic Res. 1998;28(3):323–333.

[6] Weimann A, Belling D, Poulsen HE. Quantification of 8-oxo-guanine and guanine as the nucleobase, nucleoside and deoxynucleoside forms in human urine by high-performance liquid chromatography-electrospray tandem mass spectrometry. Nucleic Acids Res. 2002;30(2):E7.

[7] Kjaer LK, Cejvanovic V, Henriksen T, et al. Cardiovascular and all-cause mortality risk associated with urinary excretion of 8-oxoGuo, a biomarker for RNA oxidation, in patients with type 2 diabetes: a prospective cohort study. Dia Care. 2017;40(12): 1771–1778.

[8] Jorgensen A, Kalliokoski O, Forsberg K, et al. A chronic increase of corticosterone age-dependently reduces systemic DNA damage from oxidation in rats. Free Radic Biol Med. 2017;104:64–74.

[9] Jorgensen A, Krogh J, Miskowiak K, et al. Systemic oxidatively generated DNA/RNA damage in clinical depression: associations to symptom severity and response to electroconvulsive therapy. J Affect Disord. 2013;149(1–3):355–362.

[10] Gedik CM, Collins A. Establishing the background level of base oxidation in human lymphocyte DNA: results of an interlaboratory validation study. FASEB J. 2005;19(1):82–84.

[11] Hah SS, Mundt JM, Kim HM, et al. Measurement of 7,8-dihydro-8-oxo-2'-deoxyguanosine metabolism in MCF-7 cells at low concentrations using accelerator mass spectrometry. Proc Natl Acad Sci USA. 2007;104(27):11203–11208.

[12] Loft S, Larsen PN, Rasmussen A, et al. Oxidative DNA damage after transplantation of the liver and small intestine in pigs. Transplantation. 1995;59:16–20.

[13] Roszkowski K, Jozwicki W, Blaszczyk P, et al. Oxidative damage DNA: 8-oxoGua and 8-oxodG as molecular markers of cancer. Med Sci Monit. 2011;17(6):CR329–33.

[14] Grew IS, Cejvanovic V, Broedbaek K, et al. Diurnal variation of urinary markers of nucleic acid oxidation. Scand J Clin Lab Invest. 2014;74(4):336–343.

[15] Bianconi E, Piovesan A, Facchin F, et al. An estimation of the number of cells in the human body. Ann Hum Biol. 2013; 40(6):463–471.

[16] Jaruga P, Dizdaroglu M. Repair of products of oxidative DNA base damage in human cells. Nucleic Acids Res. 1996;24(8): 1389–1394.

[17] Helbock HJ, Beckman KB, Shigenaga MK, et al. DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. Proc Natl Acad Sci USA. 1998;95(1):288–293.

[18] ICRP. Report of the task group on reference man. Ann ICRP. 1979;3:iii.

[19] Leinonen J, Lehtimaki T, Toyokuni S, et al. New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. FEBS Lett. 1997;417(1):150–152.

[20] Cejvanovic V, Kjaer LK, Bergholdt HKM, et al. Iron induced RNA-oxidation in the general population and in mouse tissue. Free Radic Biol Med. 2018;115:127–135.

[21] Nielsen B, Cejvanovic V, Wortwein G, et al. Increased oxidation of RNA despite reduced mitochondrial respiration after chronic electroconvulsive stimulation of rat brain tissue. Neurosci Lett. 2019;690:1–5.

[22] Sharova LV, Sharov AA, Nedorezov T, et al. Database for mRNA half-life of 19 977 genes obtained by DNA microarray analysis of pluripotent and differentiating mouse embryonic stem cells. DNA Res. 2009;16(1):45–58.

[23] Cadet J, Davies KJA, Medeiros MH, et al. Formation and repair of oxidatively generated damage in cellular DNA. Free Radic Biol Med. 2017;107:13–34.

[24] Shih BB, Farrar MD, Vail A, et al. Influence of skin melanisation and ultraviolet radiation on biomarkers of systemic oxidative stress. Free Radic Biol Med. 2020;160:40–46.

[25] Rozalski R, Siomek A, Gackowski D, et al. Substantial decrease of urinary 8-oxo-7,8-dihydroguanine, a product of the base excision repair pathway, in DNA glycosylase defective mice. Int J Biochem Cell Biol. 2005;37(6):1331–1336.

[26] Evans MD, Mistry V, Singh R, et al. Nucleotide excision repair of oxidised genomic DNA is not a source of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine. Free Radic Biol Med. 2016;99: 385–391.

[27] Chao MR, Evans MD, Hu CW, et al. Biomarkers of nucleic acid oxidation - A summary state-of-the-art. Redox Biol. 2021; 42:101872.

[28] Haghdoost S, Sjolander L, Czene S, et al. The nucleotide pool is a significant target for oxidative stress. Free Radic Biol Med. 2006;41(4):620–626.

[29] Samaranayake GJ, Troccoli CI, Zhang L, et al. The existence of MTH1-independent 8-oxodGTPase activity in cancer cells as a compensatory mechanism against on-target effects of MTH1 inhibitors. Mol Cancer Ther. 2020;19(2):432–446.

[30] Mathews CK. Deoxyribonucleotide metabolism, mutagenesis and cancer. Nat Rev Cancer. 2015;15(9):528–539.

[31] Loft S, Danielsen P, Lohr M, et al. Urinary excretion of 8-oxo-7,8-dihydroguanine as biomarker of oxidative damage to DNA. Arch Biochem Biophys. 2012;518(2):142–150.