Free Radical Research, 2002 Vol. 36 (9), pp. 929–932

Taylor & Francis healthsciences

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Commentary

DNA Repair: Insights from Urinary Lesion Analysis

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Accepted by Professor B. Halliwell

(Received 29 January 2002; In revised form 28 February 2002)

Due to various confounding factors, namely dietary contribution and cell death, measurement of urinary 8-oxo-2′-deoxyguanosine (8-oxodG) has long been considered to be no more than a marker of generalised oxidative stress. Indeed, the action of no single enzyme has been reported to excise 8-oxodG from DNA. However, analysis of recent research has suggested that these confounders may be circumvented, which, combined from work from the authors' laboratory, indicates that urinary 8-oxodG has the potential to become a most important marker of oxidative damage to, and repair of, DNA.

Keywords: Urine; Oxidative DNA damage; 8-Oxo-2′-deoxyguanosine; Antibodies; Repair

INTRODUCTION

Research interest in oxidative DNA damage, and the lesion 8-oxo-2′-deoxyguanosine (8-oxodG) in particular, has been growing at a seemingly exponential rate. Evidence for the detrimental effect that oxidative DNA damage may exert upon a cell, derives, in part, from the numerous repair systems which exist to prevent the persistence of lesions.[1,2] Indeed, defects in repair capacity are increasingly being linked to disorders such as cancer.[3] Efforts to measure DNA repair have invariably required invasive sampling procedures and a demanding methodology,[4,5] limiting their application. The appearance of lesions in urine make it a most appropriate matrix to non-invasively study *in vivo* DNA repair (Fig. 1). However, in the case of 8-oxodG, at least, ascribing urinary lesion levels to DNA repair is subject to a number of potentially confounding factors. Although recently contested,[6] diet has been shown to contribute to the urinary levels of 8-oxoguanine (8-oxoG), whilst the deoxynucleoside is unaffected,[7] hence the preference for measuring 8-oxodG. Nevertheless, in the absence of further, confirmatory studies, it would appear that the issue of diet still remains open. It has also been suggested that the majority of urinary 8-oxodG is a consequence of cell death,[8] although no experimental evidence accompanies this statement. Clearly, there would appear to be no problems with describing urinary 8-oxodG as a marker of a generalised oxidative stress, but is it possible to gain a more precise interpretation of what this lesion represents in urine?

To attempt this, the confounding factors need to be ruled out. It is generally agreed that measurement of the deoxynucleoside may overcome potential interference from the diet.[6,7] Cell death, however, is a slightly greater obstacle. Examination of the literature has revealed a number of studies measuring urinary 8-oxodG levels in patients undergoing chemotherapy with cytotoxic agents. Faure *et al.*[9] reported no increase in urinary 8-oxodG, following adriamycin (doxorubicin) treatment, despite significant increases ($p < 0.01$) in urinary uric acid, a recognised biochemical index of cell turnover. Using a similar therapy, Erhola *et al.*[10] described a trend towards a reduction in 8-oxodG levels in patients

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ISSN 1071-5762 print/ISSN 1029-2470 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1071576021000006635

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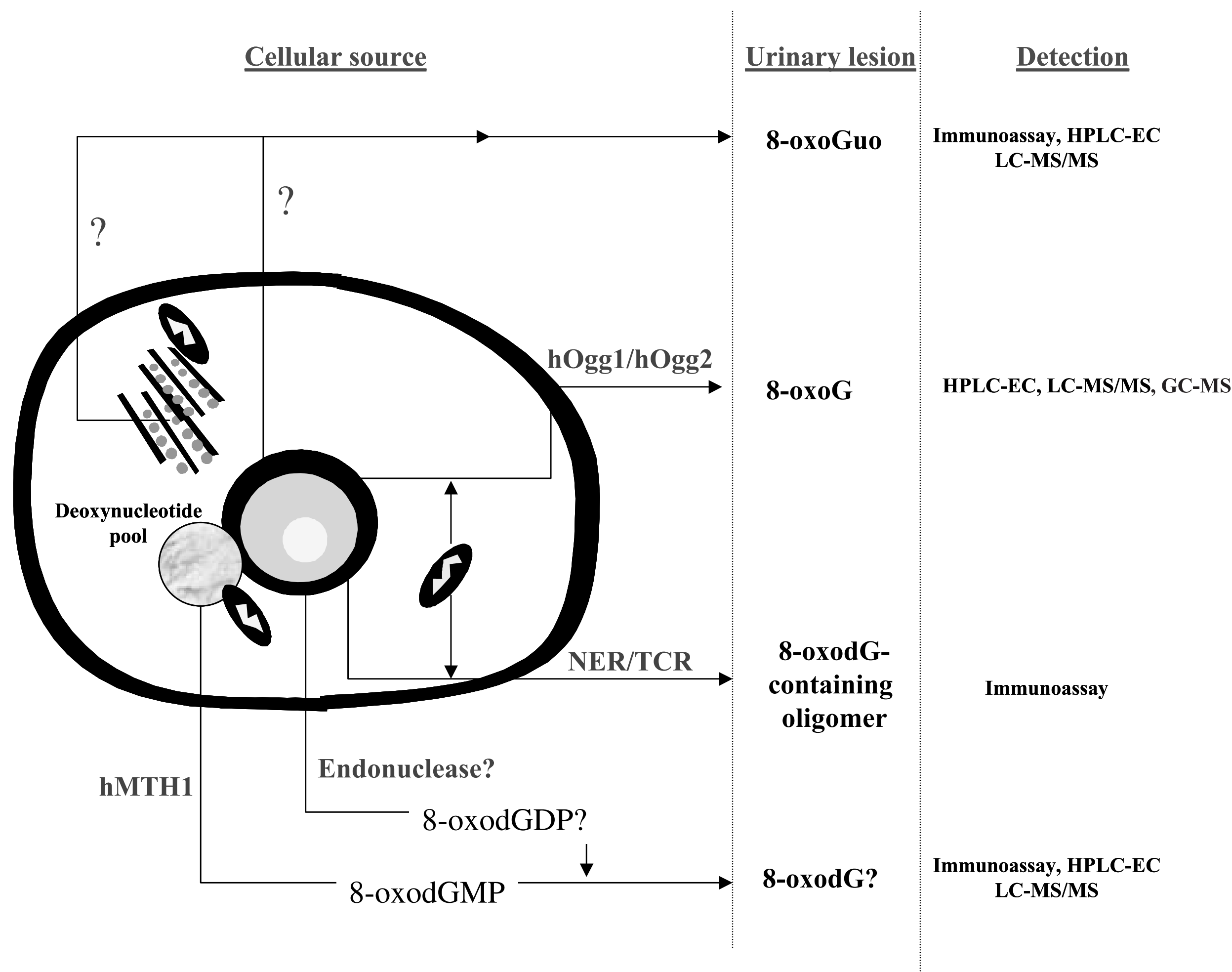

FIGURE 1 Schematic for the primary sources of extracellular, or urinary 8-oxoG/8-oxodG, and their detection. (hOgg1/hOgg2: human homologue of the Ogg enzyme; NER/TCR: nucleotide excision- and transcription-coupled repair; hMTH1: human MutT homologue; HPLC-EC: HPLC with electrochemical detection; LC-MS/MS: LC with tandem mass spectrometry; GC-MS: gas chromatography-mass spectrometry)

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showing complete or partial remission which, the authors speculated, was due to reduction in tumour mass, resulting from cell death. Equally, patients with the autoimmune disease, systemic lupus erythematosus have been reported to possess elevated rates of apoptosis[11,12] and levels of DNA in their systemic circulation,[13] whilst at the same time, urinary 8-oxodG levels are equal to, or actually less than, those seen in control subjects.[14,15] Combined, these results strongly suggest urinary 8-oxodG levels to be independent of cell death.

We have recently proposed that if cell death contributes significantly to the levels of urinary 8-oxodG, then the ratio of native to modified deoxynucleoside would be similar to that in a cell,[16] reportedly anywhere between one 8-oxodG/10^5 dG and one 8-oxodG/10^7dG.[17] Analysis of LC-MS/MS data reported by Weimann *et al.*[18] revealed an exceedingly close ratio between the two analytes (28 ± 2 8-oxodG: 12 ± 2 dG nmol/24 h), highly suggestive of a minimal contribution from cell death. It is conceivable that enzymes may exist which convert dG to G, if this is the case, the ratio between G and 8-oxoG, according to Weimann *et al.*,[18] does not even approach that seen in cells. Of course, the ratio between these two analytes might also be reduced following extensive oxidation of dG in the systemic circulation and urine, particularly given the presence of H_2O_2 in the latter.[19] Shigenaga *et al.*[20] demonstrated that dG is not subject to artefactual oxidation in such biological matrices, supported by findings from a 2-year urine storage experiment, in which levels of 8-oxodG actually decreased.[16] The literature also provides evidence that dG, through incubation with liver cytosolic enzymes or microsomal enzymes (cytochrome P-450), is not enzymically oxidised.[20] With these primary confounders addressed, attention must be focussed on the cellular

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sources of 8-oxodG. A comprehensive review of this issue listed the deoxynucleotide pool, via hMTH1, an, as yet unidentified, endonuclease, and nucleotide excision- or transcription-coupled repair (NER and TCR), as being potential sources of urinary 8-oxodG.[21] However, no single enzyme system has been identified which yields the modified deoxynucleoside.

Simultaneous analysis of urinary 8-oxodG and cyclobutane thymine dimers, following UV exposure of volunteers, revealed coincident peaks of excretion, suggestive of a common repair process, speculated to be NER or TCR.[22] Recent work from our laboratory, using a combination of antibodies to single-stranded DNA and DNA lesions has suggested that a proportion of 8-oxodG appears in the urine in the form of oligomers.[23] We reported how changes in cell culture medium levels of DNA oligomers showed close similarity with 8-oxodG levels following vitamin C supplementation of cells.[23] This was supported *in vivo* with correlations between urinary oligomer, thymine dimer and 8-oxodG levels. This study, largely based upon correlations, has recently been supported by new findings revealing a dichotomy in urinary 8-oxodG levels following vitamin C supplementation, depending upon whether HPLC-EC or ELISA methods are used.[16] These data suggest the dichotomy derives from HPLC-EC detecting only the modified deoxynucleoside, whereas ELISA measures 8-oxodG, not only as the deoxynucleoside, but also within oligomers. Given the proposed rôle of vitamin C in the upregulation of DNA repair,[24,25] these results indicate a relationship between DNA repair and lesion-containing oligomers. Furthermore, experiments examining the excretion of 8-oxodG from NER proficient and deficient cell lines, demonstrated extracellular 8-oxodG, in the absence of any cell death.[25] From such studies we suggest a number of, potentially crucial, proposals:

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i) The ratio of native to modified deoxynucleoside in urine, and studies reporting the absence of increases in urinary 8-oxodG after treatment with cytotoxic agents, implies that cell death does not contribute significantly to levels of urinary 8-oxodG.
ii) Such techniques as HPLC-EC and LC-MS/MS which, by the nature of their procedure, measure urinary 8-oxodG as the free deoxynucleoside, do not take into account lesions contained within oligomers (Fig. 1).
iii) Measurement of urinary 8-oxodG, or indeed any lesion, within oligomers, is reflective of NER or TCR.
iv) If, as we have recently indicated, vitamin C modulates the removal of 8-oxodG by NER or TCR,[25] only the measurement of urinary 8-oxodG-containing oligomers will reflect changes in NER/TCR activity towards 8-oxodG.

After 13 years of being assessed as a generalised marker of oxidative stress, research is beginning to dissect the processes which remove 8-oxodG such that its measurement in urine can be imparted with greater significance.

Acknowledgements

The authors wish to acknowledge financial support from the Ministry of Agriculture, Fisheries and Food, Food Standards Agency, Arthritis Research Campaign and Leicester Dermatology Research Fund.

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