Acta Biochimica Polonica

Vol. 50 No. 2/2003

549–553

QUARTERLY

Communication

Cellular level of 8-oxo-2′-deoxyguanosine in DNA does not correlate with urinary excretion of the modified base/nucleoside✪

Marek Foksinski, Daniel Gackowski, Rafał Rozalski and Ryszard Olinski✉

Department of Clinical Biochemistry, The Ludwik Rydygier Medical University in Bydgoszcz, Bydgoszcz, Poland

Received: 12 February, 2003; revised: 21 May, 2003; accepted: 30 May, 2003

Key words: 8-oxo-2′-deoxyguanosine, 8-oxoguanine, oxidative DNA damage

We assessed a relationship between the level of 8-oxodG in leukocyte DNA measured with the high performance liquid chromatography with electrochemical detection (HPLC/EC) technique and urinary excretion of the modified nucleoside/base analysed with a recently developed methodology involving HPLC prepurification followed by gas chromatography with isotope dilution mass spectrometric detection. No correlation was found between these markers of oxidative DNA damage commonly used in epidemiological studies. Several possible explanations of this finding are discussed.

In recent years thorough qualitative and quantitative characterization of oxidative DNA base modifications has attracted particular interest since some of them may play a significant role in various stages of carcinogenesis. 8-Oxoguanine (8-oxoGua) is one of the most critical lesions of this type. The presence of 8-oxoGua residues in DNA leads to a GC to TA transversion unless repaired prior to DNA replication (Cheng *et al.,* 1992). Therefore, the presence of 8-oxoGua may lead to mutagenesis. Furthermore, many observations indicate a direct correlation between 8-oxoGua formation and carcinogenesis *in vivo* (Feig *et al.,* 1994; Floyd, 1990).

Background levels of 8-oxoGua have been analysed in different human tissues (Kasai, 1997). However, direct measurements of the

✪This work was financed by a grant from The Ludwik Rydygier Medical University in Bydgoszcz (BW 6/2001).

✉Address for correspondence: Ryszard Olinski, Department of Clinical Biochemistry, Karlowicza 24, 85-092 Bydgoszcz, Poland; tel.: (48 52) 585 3770; fax: (48 52) 585 3771; e-mail: ryszardo@amb.bydgoszcz.pl

Abbreviations: dG, 2′-deoxyguanosine; 8-oxodG, 8-oxo-2′-deoxyguanosine; 8-oxoGua, 8-oxoguanine

level are compromised by the limitations of current methodologies and variation between different cell types (Collins, 1999; ESCODD, 2000; ESCODD, 2002a; ESCODD, 2002b; Gackowski *et al.,* 2001a). An alternative approach to asses oxidative DNA damage is the measurement of urinary excretion of 8-oxoGua and 8-oxo-2′-deoxyguanosine (8-oxodG). It is generally accepted that the products of repair of 8-oxoGua in cellular DNA are excreted into the urine without further metabolism (Cooke *et al.,* 2000; Loft & Poulsen, 1998; Shigenaga *et al.,* 1989) and that the amount of the modified base/nucleoside excreted into urine should represent the average rate of DNA damage in the whole body. Using a recently developed methodology involving HPLC prepurification followed by gas chromatography with isotope dilution mass spectrometric detection, we found that urinary excretion of 8-oxoGua and 8-oxodG does not depend on diet in the case of humans and may reflect the involvement of different repair mechanisms, namely BER (base excision repair) and NER (nucleotide excision repair) (Gackowski *et al.*, 2001b).

White blood cells are often used as surrogate cells which should inform about the oxidative stress — measured as a certain level of 8-oxodG — in other tissues (Collins *et al.,* 1998; Lenton *et al.,* 1999). Therefore, in the present study we assessed the relationship between the level of 8-oxoGua in leukocyte DNA measured with the HPLC/EC technique and urinary excretion of the modified nucleoside/bases analysed with the above described methodology. The aim of this work was to find out whether a correlation exists between the results of two the approaches that are currently used to asses oxidative DNA damage.

MATERIALS AND METHODS

Subjects. The study was conducted in a group consisting of 81 human subjects; males (n = 66) and females (n = 15) with an age range of 44 to 83 years. This group comprised 20 healthy smokers and 61 non-small cell lung cancer patients. Blood samples and 24-h urine samples were collected. The study was approved by the medical ethics committee of The L. Rydygier Medical University, Bydgoszcz, Poland, No. 188/2001 (in accordance with Good Clinical Practice, Warsaw, 1998) and all the patients gave informed consent.

Isolation of leukocytes from venous blood. Blood from venous blood samples was carefully applied on top of Histopaque 1119 solution (Sigma) and leukocytes were isolated by centrifugation according to the procedure laid down by the manufacturer.

DNA isolation and 8-oxodG determination. DNA was isolated from leukocytes using the method described by Miller *et al.* (1988) with some modifications (Gackowski *et al.*, 2002). Determination of 8-oxodG by means of the HPLC/EC technique was described previously (Foksinski *et al.*, 1999).

Urine analysis. To 2 ml of human urine were added 0.5 nmol of [$^{15}N_3$,^{13}C]8-oxoGua, 0.05 nmol of [^{18}O]8-oxodG and 10 μl of acetic acid (Sigma, HPLC grade). The isotopic purity of the standards was 97.65% and 96.5%, respectively. After centrifugation (2000 × ***g***, 10 min), supernatant was filtered through a Millipore GV13 0.22 μm syringe filter and 500 μl of this solution was injected into an HPLC system. In a pilot study isotopically labeled internal standards of unmodified compounds (1 nmol of [$^{13}C_3$]guanine and 1 nmol of [$^{15}N_5$]2′-deoxyguanosine) were added to the urine samples to monitor fractions containing both these compounds and to avoid overlapping of the peaks containing the modified and unmodified base/nucleoside. The isotopic purity of the standards was 96.4% and 98.0%, respectively.

Urine HPLC purification of 8-oxoGua and 8-oxodG was performed according to the method described by Gackowski *et al.* (2001b; Rozalski *et al.,* 2002).

GC/MS analysis was performed according to the method described by Dizdaroglu (1994),

adapted for additional [^{18}O]8-oxoGua analyses (the *m/z* 442 and 457 ions were monitored).

RESULTS AND DISCUSSION

In the present trial no significant correlation was found between the two biomarkers that are most commonly used to determine oxidative DNA damage: the level of 8-oxodG in leukocyte DNA and of urinary excretion of the modified base/nucleoside (Fig. 1). This lack of correlation was detected in healthy subject as well as in the patient group. There are several possible explanations of these results:

- the amount of the modified base/nucleoside excreted into urine should represent the average rate of DNA damage in the whole body (Cooke *et al.,* 2000; Loft & Poulsen, 1998) and the level of 8-oxodG in leukocyte DNA does not necessarily reflect the damage in other tissues.

To the best of our knowledge there have been no experimental data to date which prove the existence of a relationship between leukocytes and other tissues concerning oxidative DNA damage. Factors responsible for the formation of 8-oxodG and for oxidative stress may vary in different tissues. For example such factors as the activity of key antioxidant enzymes – superoxide dismutase, catalase and glutathione peroxidase, which may influence oxidative stress, vary in different organs (Halliwell & Gutteridge, 1999). Differences in metabolic rate may influence reactive oxygen species production (Loft *et al.,* 1994) and these differences may also depend on the tissue. Our earlier results demonstrate that there is no relationship between myometrial tissues and the lymphocytes concerning the level of 8-oxodG (Foksinski *et al.,* 2000). We did not find such a correlation between leukocytes of lung cancer patients and lung cancer tissue or the marginal tissue free of cancer (unpublished results). Also studies on rats are consistent with the existence of different levels of 8-oxodG in different tissues (Devanaboyina & Gupta, 1996; Nakae *et al.,* 2000).

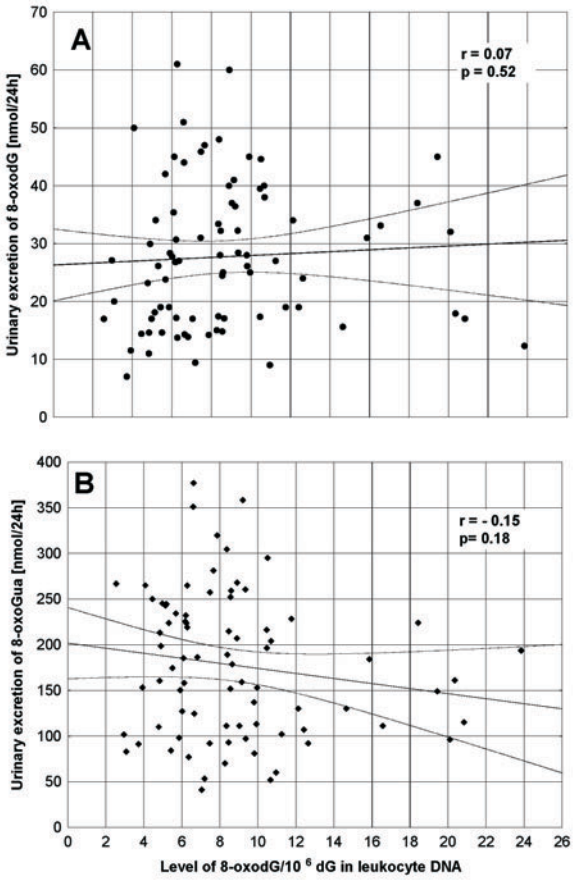

Figure 1. Correlation coefficients between the level of 8-oxodG in leukocyte DNA and:

A, urinary excretion of 8-oxodG; B, urinary excretion of 8-oxoGua. Urine samples were collected day and night (24 h output).

- The levels of oxidative DNA damage represent a dynamic equilibrium between oxidative DNA damage and repair of the damage in the specific tissue/cells while, as it was mentioned above, the level of 8-oxoGua and 8-oxodG (modified base/nucleoside) in urine may be an indicator of oxidative DNA insult, and a general index of oxidative stress at the level of the organism. It should be also mentioned that a correlation between the level of 8-oxodG in cellular DNA and urinary excretion of the repair products

can be expected only in the case of a constant repair rate of the lesion.

- We cannot entirely exclude the possibility that processes other than DNA repair can contribute to 8-oxoGua and 8-oxodG level in human urine, e.g. 8-oxodG may derive from dead cells (Lindahl, 1993) or from sanitation of the cellular nucleotide pool by a MutT directed pathway (Hayakawa *et al.,* 1995) and the excretion of 8-oxoGua may also include a contribution from oxidized RNA, particularly if mechanisms exist to maintain the integrity of RNA molecules (but such a mechanism(s) has not been detected yet).

Under physiological conditions unmodified 2′-deoxoguanosine is decomposed by cellular purine nucleoside phosphorylase (PNP-ase) that catalyses phosphorolysis of N-glycosidic bond to generate free guanine and deoxoribose 1′-monophosphate. However, a commercial preparation of rabbit PNP-ase is totally inactive towards 8-oxodG (unpublished result). Therefore, it is rather unlikely that the 8-oxoGua observed in urine comes from degradation of 8-oxodG *via* this pathway.

In conclusion, we did not find a correlation between 8-oxodG level in leukocyte DNA and urinary excretion of the modified base/nucleoside. It is also noteworthy that no correlation was found between either of the investigated parameters (urinary 8-oxoGua, 8-oxodG and 8-oxodG level in leukocyte DNA) and the age of the subjects. However, it is encouraging that in cancer patients an increased oxidative DNA damage can be detected in blood cells and in urine in comparison with healthy subjects (Olinski *et al.,* 2002; Rozalski *et al.,* 2002).

REFERENCES

Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA. (1992) *J Biol Chem.*; **267**: 166–72.

Collins AR. (1999) *Bioessays.*; **21**: 238–46.

Collins AR, Gedik CM, Olmedilla B, Southon S, Bellizzi M. (1998) *FASEB J.*; **12**: 1397–400.

Cooke MS, Evans MD, Herbert KE, Lunec J. (2000) *Free Radic Res.*; **32**: 381–97.

Devanaboyina U, Gupta RC. (1996) *Carcinogenesis.*; **17**: 917–24.

Dizdaroglu M. (1994) *Methods Enzymol.*; **234**: 3–16.

ESCODD. (2000) *Free Radic Res.*; **32**: 333–41.

ESCODD. (2002a) *Carcinogenesis.;* **23**: 2129–33.

ESCODD. (2002b) *Free Radic Res.;* **36**: 239–45.

Feig DI, Reid TM, Loeb LA. (1994) *Cancer Res.*; **54**: 1890s–4s.

Floyd RA. (1990) *Carcinogenesis.*; **11**: 1447–50.

Foksinski M, Bialkowski K, Skiba M, Ponikowska I, Szmurlo W, Olinski R. (1999) *Mutat Res.*; **438**: 23–7.

Foksinski M, Kotzbach R, Szymanski W, Olinski R. (2000) *Free Radic Biol Med.*; **29**: 597–601.

Gackowski D, Ciecierski M, Jawien A, Olinski R. (2001a) *Acta Biochim Polon.*; **48**: 535–9.

Gackowski D, Rozalski R, Roszkowski K, Jawien A, Foksinski M, Olinski R. (2001b) *Free Radic Res.*; **35**: 825–32.

Gackowski D, Kruszewski M, Bartlomiejczyk T, Jawien A, Ciecierski M, Olinski R. (2002) *J Biol Inorg Chem.*; **7**: 548–50.

Halliwell B, Gutteridge JM. (1999) Oxford University Press, New York, NY.

Hayakawa H, Taketomi A, Sakumi K, Kuwano M, Sekiguchi M. (1995) *Biochemistry.*; **34**: 89–95.

Kasai H. (1997) *Mutat Res.*; **387**: 147–63.

Lenton KJ, Therriault H, Fulop T, Payette H, Wagner JR. (1999) *Carcinogenesis.*; **20**: 607–13.

Lindahl T. (1993) *Nature.*; **362**: 709–15.

Loft S, Poulsen HE. (1998) *Acta Biochim Polon.*; **45**: 133–44.

Loft S, Astrup A, Buemann B, Poulsen HE. (1994) *FASEB J.*; **8**: 534–7.

Miller SA, Dykes DD, Polesky HF. (1988) *Nucleic Acids Res.*; **16**: 1215.

Nakae D, Akai H, Kishida H, Kusuoka O, Tsutsumi M, Konishi Y. (2000) *Lab Invest.*; **80**: 249–61.

Olinski R, Gackowski D, Foksinski M, Rozalski R, Roszkowski K, Jaruga P. (2002) *Free Radic Biol Med.*; **33**: 192–200.

Rozalski R, Gackowski D, Roszkowski K, Foksinski M, Olinski R. (2002) *Cancer Epidemiol Biomarkers Prev.*; **11**: 1072–5.

Shigenaga MK, Gimeno CJ, Ames BN. (1989) *Proc Natl Acad Sci U S A.*; **86**: 9697–701.