chapter thirteen

Interpretation of oxidative DNA modification: relation between tissue levels, excretion of urinary repair products

Henrik E. Poulsen and Stefen Loft

13.1 INTRODUCTION

The oxidative modifications in DNA (Dizdaroglu 1998, Halliwell and Aruoma 1991) can be measured in tissue samples by extraction and hydrolysis of the DNA (Kasai *et al* 1986), and DNA repair products after oxidative modifications can be measured in urine (Adelman *et al* 1988, Ames 1989, Cathcart *et al* 1984, Loft *et al* 1992, Tagesson *et al* 1992). Oxidative modifications in DNA can also be measured in single cells by the Comet assay as discussed by Collins (1998) in this volume. These methodologies facilitate use of different approaches and designs for in depth studies of the oxidative modification of DNA, its relation to pathophysiological processes and diseases and the effect of antioxidants.

The hydroxylation in the 8-position of guanine in DNA (8-oxoGua, the corresponding nucleoside designated 8-oxodG) is the most abundant oxidative modification observed and studied. The most common methodologies are high pressure liquid chromatography with electrochemical detection (HPLC-EC), gas-chromatography mass spectroscopy and ^{32}P-postlabeling. The levels reported by the two latter techniques show a large variation with values ranging 100 fold. By HPLC-EC levels for 8-oxodG are generally reported about 1×10^{-5} dG. It has been argued that sample preparation

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procedures for gas-chromatography can produce oxidative base profile in DNA (Fuciarelli *et al* 1989, Ravanat *et al* 1995, Aruoma *et al* 1989, Jenner *et al* 1998) and there might be problems with certain extractions and efficiency of the DNA digesting enzymes used.

13.2 Interpretation of Tissue Levels of DNA-Modification and Urinary Excretion of DNA Repair Products

The level of oxidized DNA bases in a tissue is determined by the rate by which DNA is oxidized and the rate by which oxidative modifications are removed. In studies of 8-oxodG in tissues from humans in a broad age range no sign of major accumulation are apparent (Bartsch *et al* 1991, Bergtold *et al* 1990, Loft and Poulsen 1996) and unless there are sudden changes in the oxidative stress within a defined time period the level of oxidized bases in DNA appears constant. As mentioned earlier there are no apparent indications of major accumulation in human tissue. In human cells induced high levels of oxidized nucleobases return to initial values within some hours (Jaruga and Dizdaroglu 1996). Further support for the concept of a steady state is the relatively low variation in individual urinary excretions of 8-oxodG in subjects and control group in intervention groups of a controlled trial (Prieme *et al* 1997) and a very low intra-individual variation, 22%, in a carefully controlled intervention trial (Verhagen *et al* 1995), also because 8-oxodG is rapidly excreted into urine after injection (Loft *et al* 1995). In lung cancer tissue (Jaruga *et al* 1994), renal cancer tissue (Okamoto *et al* 1994) and in lymphocytes from patients with autoimmune disease (Bashir *et al* 1993) levels of oxidized nucleobases have been reported somewhat different from control values, whereas no difference was observed in breast cancer and prostatic hyperplasia (Nagashima *et al* 1995, Olinski *et al* 1995). It is difficult to find group differences larger that one fold, the only considerable deviation from control value is the 10x reported increase in lymphocytes from patients with Fanconi's anaemia (Degan *et al* 1995), even radiation does not produce impressive increases in DNA oxidation *in vivo* (Mori *et al* 1993).

Collectively, these data suggest a steady state level of oxidized nucleobases in nuclear DNA that can be modified by doubling or halving, except in very special disease states where larger deviations have been demonstrated. Yet, the variation between individuals is much larger.

This urinary excretion of oxidized nucleosides has been advocated as a possible assay for oxidative DNA damage (Cathcart *et al* 1984). The amounts of 8-oxodG excreted into urine are reported without much variation between various laboratories, for review see (Loft and Poulsen 1996), individual variation is 7 fold, with main part of the values ranging 200–600 nmol per kg body weight per 24 h (14–60 pmol/70 kg per 24 h. The genome contains 3×10^9 base pairs, the calculated hit rate producing 8-hydroxylation of guanine is 158–504 hits per day. If no repair was functioning a doubling of the number of oxidized nucleobases would take about 60 to 190 days and 1% of the genome would be oxidized within 8 years.

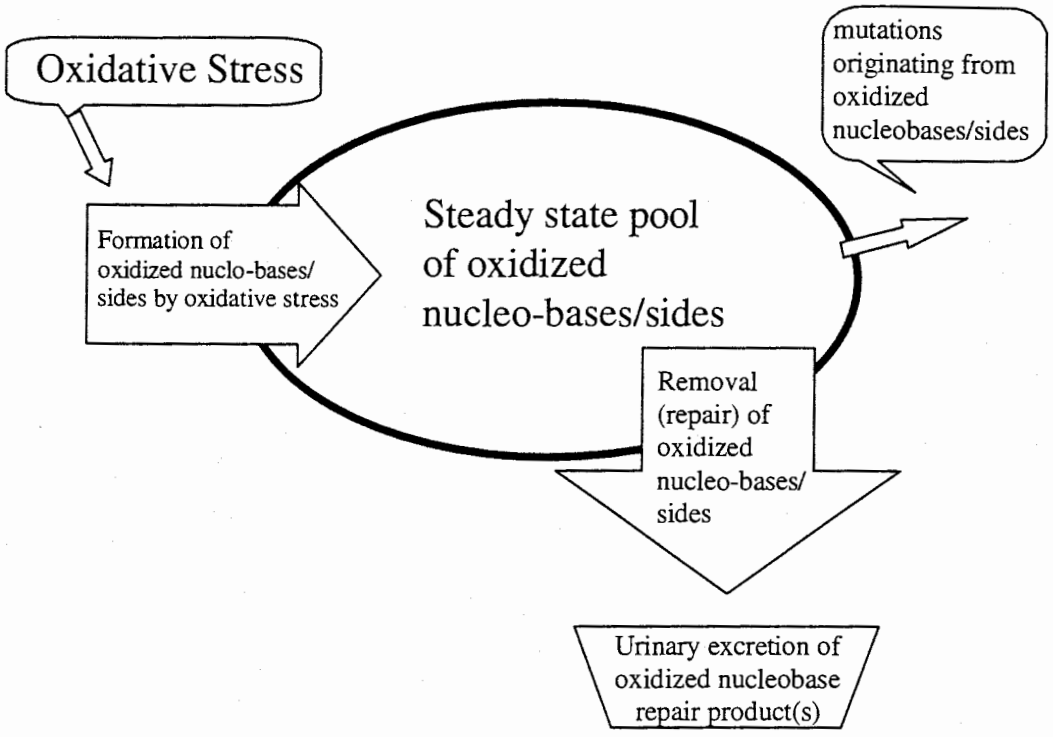

Figure 13.1 Simplified scheme for formation of oxidized nucleobases in the cellular DNA pool, removal of oxidized nucleobases by repair and mutagenesis.

Several points can be inferred from these observations. Repair must be extensive and efficient since a steady state concentration of 8-oxodG in nuclear DNA is maintained, as clearly demonstrated in the classical study of Kasai *et al* (1986). Considering the situation depicted in Figure 13.1, nuclear DNA can be considered a pool of oxidized nucleobases where there exist a constant output (urinary excretion) which must correspond to the input, i.e. formation of new oxidized nucleobases. A small proportion of oxidized nucleobases, particularly 8-hydroxylated guanine (Emmert *et al* 1995, Kuchino *et al* 1987, Retèl *et al* 1993), leads to mutational events. Removal of modified bases by this pathway can be considered without importance for the removal process although highly important for carcinogenesis. The pool includes not only modified bases in nuclear DNA but also modified bases in mitochondria and modified nucleosides and bases in the extranuclear-extramitochondrial confinement. Repair of nuclear and mitochondrial DNA have to be almost 100% efficient and in that situation the excretion rate can be considered independent of repair. In mitochondria several DNA copies are present and some may be removed without repair. Even if there are some changes in repair rate (or removal rate) this will not influence this argument. Consequently, the urinary excretion of oxidized nucleosides is a measure of the rate of oxidation of DNA. It should be noted that the urinary excretion represents the cumulated body burden and that it cannot be determined if this originated from an impact to

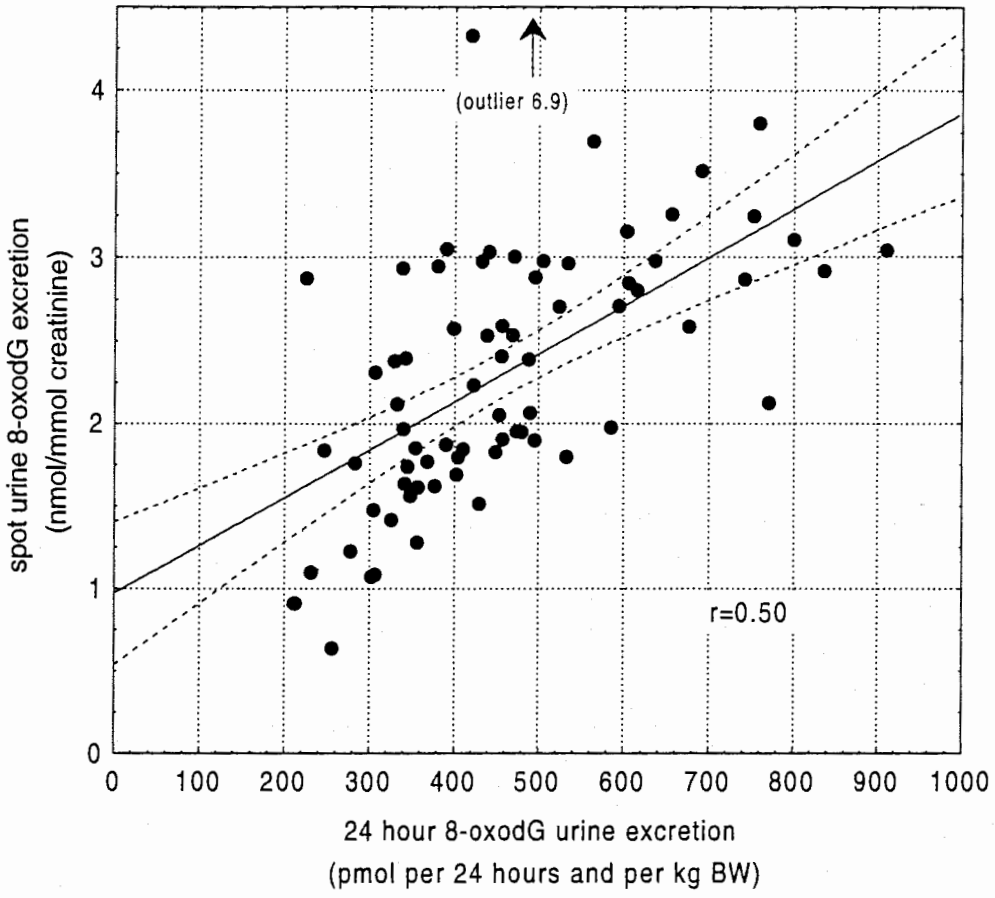

Figure 13.2 Relation between 8-oxodG in 24 hour urine sample (nmol/24 h/kg BW) and 8-oxodG in spot urine, following morning, corrected for creatinine concentration (nmol/mmol creatinine) in 74 persons. The correlation coefficient is 0.50. Samples originate from at previous study (Loft *et al* 1992).

all body cells or to much higher insult to several organs. Interpretation of urinary excretion of oxidized bases is more difficult since it depends also on dietary intake (Shigenaga *et al* 1989).

In contrast, tissue levels represent the measure of a concentration, reflecting the balance between rate of oxidation and the rate of repair. The major interpretation of a change in urinary nucleoside excretion is a change in the rate of oxidative stress. A change in tissue concentration of oxidized nucleobases/nucleosides cannot with certainty be related to change in oxidative stress, to change in repair, or to a combination.

The concentration measurement can be related to the relevant nucleobase, (in based products) e.g. 8oxodG (or 8oxoGua) per 10^{-5} dG (or Gua). It is also customary to give the concentration measure related to measurement of DNA by the UV differential method. It is also relevant to relate the oxidized base measurement to the corresponding unmodified base.

Collection of urine for 24 hours or longer is quite straight forward, however, it may present some practical problems. To alleviate such practical problems some investigators have used the urinary creatinine as a means of correcting for shorter urinary collection periods or spot urine, the idea being that urinary creatinine excretion is independent of the urinary production. Figure 13.2 shows the levels of 8-oxodG in urine collected for 24 hour and an initial spot urine collection related to that period in the same individual. There is a correlation of 0.50 between the creatinine corrected spot urine and 24 hour urine 8-oxodG excretion. This means that for repeated measurements in the same individual the creatinine correction may be applied. However, for comparison of e.g. a diseased group with a control group differences in e.g. muscle mass where much creatinine is found, the creatinine corrected of spot urine 8-oxodG excretion may be difficult to relate to the disease, rather it reflects difference in creatinine excretion.

13.3 Relation Between Experimentally Induced 8-oxodG in Tissues and Urinary Excretion

In an experimental study, oxidative stress was induced by the administration of the hepatocarcinogen 2-nitropropane, 100 mg/kg, to rats (Deng *et al* 1998). Table 13.1 gives the levels of 8oxodG in liver, kidney and bone marrow. In the animals sacrificed 6 and 24 hours after 2-nitropropane administrations an increase of 33% (95% CL 9–57%) and 43% (95% CL 27–60%) of the 24 hours urinary 8-oxodG excretion before the treatment (16822 pmol/24 hour) was observed. Assuming 1) that the liver represent about 3.5% of the body mass, 2) that the kidneys and bone marrow represent another 3% of the body mass, 3) the 6 hour concentration of 8-oxodG in these organs is close to the peak values induced, 4) that 2-nitropropane induces a short and distinct increase in oxidative stress without interfering with DNA repair, and 5) that other parts of the body do not show increased oxidative stress, it is possible to relate the increase in these organs to the increase in the urinary excretion. The liver represents 3.5% of body mass increased 8 fold, which contributes 28% of total body burden. The kidney and bone marrow represent 3% of body mass increased 4 fold, which gives 12% contribution to total body burden. This totals 40% of total body burden and corresponds to 43% increased urinary output of 8-oxodG. Such calculations are of course subject to high variation, however it is interesting that the numbers are so consistent with the idea that 2-nitropropane induces a distinct oxidative insult to organs that are able to metabolize the substance followed by repair and urinary excretion within a short time, *in casu* 24 hours.

Table 13.1 The 8-oxodG concentration (per 10^5dG) in various rat liver, kidney and bone marrow after administration of 2-nitropropane (100 mg/kg body weight i.p.)

	control	6 hours	24 hours
Liver	1.00 ± 0.42	8.68 ± 4.23	2.94 ± 1.10
Kidney	0.93 ± 0.19	2.04 ± 0.42	1.39 ± 0.39
Bone Marrow	1.04 ± 0.50	nd	5.14 ± 2.42

13.4 Relation Between Direct and Indirect Measurements of Oxidative DNA Modification

The direct measurement of oxidative modifications in DNA by HPLC-EC, GC-MS or ^{32}P-postlabeling techniques is very specific and extensively discussed in this volume. The "Comet" assay has been advocated as a measure of DNA damage (Ostling and Johanson 1984). The assay requires only a few hundred cells that are placed on a mini agarose gel and lysed for electrophoresis under alkaline conditions. Oxidative modifications and strands-breaks in DNA release its supercoiling and allow the uncoiled DNA to extend towards the anode. Visualized by a fluorescent DNA dye and fluorescent microscopy the relaxed DNA extension looks like a comet. Various quantitation methods can be used (Collins *et al* 1993, Duthie *et al* 1996, Ross *et al* 1995, Tuo *et al* 1996a, b). The assay can be expanded by the use of specific DNA repair enzymes, that will induce strand breaks e. g. at endonuclease sensitive sites and can be interpreted as pyrimidine modification.

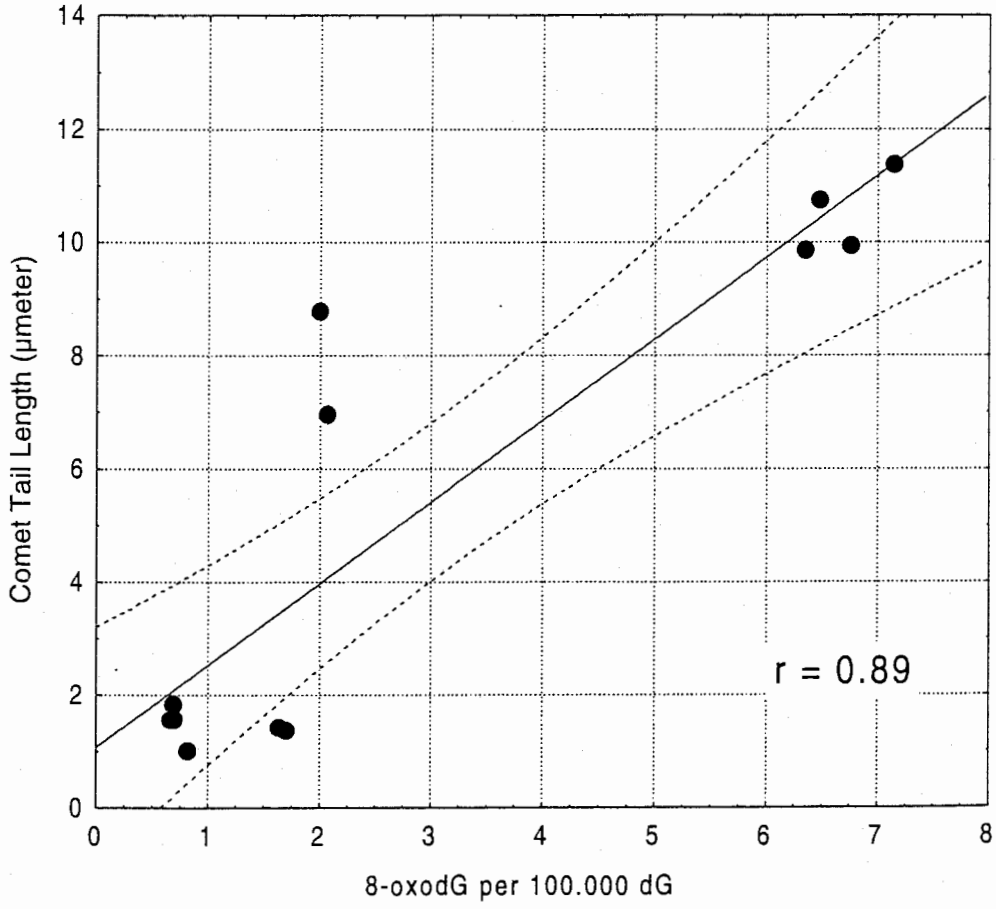

Figure 13.3 The effect of administration of 2-nitropropane (100 mg/kg) on hepatic, renal and bone marrow concentrations of 8-oxodG. Each value represent the average ± SD of groups of 12–16 rats.

Figure 13.3 depicts a previous study where we measured both the concentration of 8-oxodG and the Comet tail length in rats with and without oxidative stress induced by i.p. administration of the hepatotoxin 2-nitropropane. The correlation between these two measures is high, r = 0.89 (Deng *et al* 1998). In the study the technique with specific repair enzyme to induce strand breaks at sensitive sites was not used. The measurement of 8-oxodG concentration is specific for that lesion and the high correlation between 8-oxodG and the Comet tail length suggests that 8-hydroxylation of guanine induces relaxation of DNA super coiling or that other damage occurs in parallel also from a quantitative point of view. The Comet assay is not specific to the same extent as the chemical measurement of 8-oxodG and must be assumed to measure any change in DNA that induce changes in the super-coiling structure. It is therefore possible that under other experimental settings there is not necessarily a similar high correlation (see Collins 1998).

Along these lines it is interesting that antioxidant intervention in smokers decreases the oxidative damage in lymphocytes measured by the Comet assay (Duthie *et al* 1996), whereas in another study intervention with single antioxidants did not influence the rate of oxidative modification measured by urinary excretion of 8-oxodG (Prieme *et al* 1997). There are several differences in the design of the two intervention studies. This makes it difficult to determined with certainty which factor(s) are responsible for the differences. Determination of the factor(s) responsible will require e.g. a study where several different measures of oxidative stress and repair are included.

13.5 Conclusions

Oxidative modification of DNA caused by endogenous or exogenous oxidative stress may be significant in the aetiology of cancer (Ames 1983, 1989, Beckman and Ames 1998 in this volume). Methodologies for measuring DNA modification by oxidation include determination by highly specific analytical methods applied to tissue samples or to surrogate target cells, e.g. peripheral lymphocytes, to less specific measurements of changes in the super coiling structure of DNA using alkaline electrophoresis of single cells and measurements of urinary excretion of DNA repair products.

The urinary excretion of DNA repair products is a measure of the rate of DNA oxidation and is, for practical purposes independent of DNA repair. It should be stressed that correction for incomplete or very short sampling, i.e. spot sampling, and correction by urinary creatinine concentration is a questionable method that under the best circumstances has a correlation coefficient 0.5. Comparing groups with different muscle mass, and thus different creatinine excretion will lead to severe difficulties in the interpretation of spot urine 8-oxodG excretion (Poulsen *et al* 1996).

Direct chemical measurement of the oxidative modification in DNA, e.g. as number of specifically oxidized bases/nucleosides, in cells or tissue is a concentration measure reflecting the balance between rate of oxidation and rate of repair. As such it is substantially different from measurements of the urinary excretion of repair products. Indirect methods to determine oxidative modification of DNA include the alkaline Comet assay. Under controlled experimental conditions it is possible to establish clear

relations between these different methodologies for estimating oxidative stress to DNA, and clearly they provide different albeit important information.

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