Pergamon

European Journal of Cancer Vol. 31A, No. 6, pp. 934–940, 1995

0959–8049/95 $9.50 + 0.00

0959-8049(94)00490-0

Determination of Urinary 8-Hydroxydeoxyguanosine by Automated Coupled-column High Performance Liquid Chromatography: A Powerful Technique for Assaying *In Vivo* Oxidative DNA Damage in Cancer Patients

C. Tagesson, M. Källberg, C. Klintenberg and H. Starkhammar

An automated analytical method has been developed for determination of the oxidative DNA adduct, 8-hydroxydeoxyguanosine (8OHdG) in human urine, based on coupled-column high performance liquid chromatography with electrochemical detection. Urine is concentrated on Bondelut CH by means of an automated sample processor, and the enriched sample injected on to a polymeric reversed phase column coupled in line with an electrochemical detector and a C_{18} reversed phase column. By use of the electrochemical detector, a suitable retention time interval is set for collection of the fraction containing 8OHdG from the chromatography on the first column; this fraction is collected in a 2 mL loop and injected onto the C_{18} column. The system is operated by an automatic valve station controlled by an integrator. The method has a large sample capacity and measures 31.1, 15.7, and 7.43 nmol 8OHdG/L urine with variation coefficients of 8, 8 and 24% within series and 8, 11 and 23% between series. Normal healthy individuals were found to excrete 14.9 ± 7.8 nmol 8OHdG/24 h, or 1.11 ± 0.62 μmol 8OHdG per mol creatinine, in their urine, whereas increased levels of 8OHdG were found in 24 h collections from a variety of cancer patients, both in samples taken before onset of oncological therapy (1.84 ± 1.12 μmol/mol creatinine, $P < 0.01$ versus healthy individuals) and after therapy onset (2.18 ± 1.44 μmol/mol creatinine, $P < 0.001$ versus healthy individuals). Moreover, mean values of 8OHdG in random urinary samples from cancer patients were significantly higher than from healthy individuals (2.42 ± 2.28 versus 1.19 ± 0.48 μmol/mol creatinine, $P < 0.001$), both in samples taken before therapy onset (1.91 ± 0.96, $P < 0.001$ versus healthy individuals) and after (2.57 ± 2.46, $P < 0.001$ versus healthy individuals). High levels of urinary 8OHdG were found in patients subjected to whole body irradiation, and in patients receiving chemotherapy with various cytostatic agents. The potential use of the method for detecting increased urinary 8OHdG excretion and conditions associated with increased oxidative DNA damage in humans is discussed.

Key words: free radicals, oxidative DNA damage, urinary 8-hydroxydeoxyguanosine, coupled-column HPLC, irradiation, cytostatic agents

Eur J Cancer, Vol. 31A, No. 6, pp. 934–940, 1995

INTRODUCTION

OXIDATIVE DAMAGE to DNA has been suggested to contribute to a number of diseases including cancer and chronic inflammation [1–6]. In order to study the relationship between oxidative damage to DNA and diseases, it is desirable to develop techniques which can be used for the analysis of DNA damage products in individuals. Analytical approaches, such as gas chromatography – mass spectrometry (GC–MS) and immunochemical techniques, have been shown to be effective methods for detecting a number of specific lesions caused by oxidative damage, including the modified bases 8-hydroxyguanine, 8-hydroxyadenine and thymine glycol [7–9]. In general, these methods are useful for detecting high levels of oxidative DNA damage but have, as yet, not been used for measuring the lower levels of damage that may occur in humans. Previous efforts aimed at quantitating endogenously produced oxidative DNA damage have involved measuring the urine levels of thymine glycol and thymidine glycol by HPLC combined with UV

Correspondence to C. Tagesson.
C. Tagesson is at the Department of Occupational and Environmental Medicine, M. Källberg is at the Department of Clinical Chemistry; C. Klintenberg and H. Starkhammer are at the Department of Oncology, Faculty of Health Sciences, Linköping University, S–581 85 Linköping, Sweden.
Revised 20 Sep. 1994; accepted 3 Nov. 1994.

detection [10]. However, this method has certain limitations, principally the poor sensitivity and long time required to perform the assay.

The problem associated with detecting low levels of oxidatively damaged DNA bases has recently been resolved by the identification of another oxidised DNA-adduct, 8-hydroxydeoxyguanosine (8OHdG) [11]. This adduct can be quantitated with a high degree of sensitivity and selectivity by electrochemical detection following separation of enzymatic hydrolysates of DNA by reversed phase HPLC [12]. The electrochemical detection of 8OHdG is about 1000 times more sensitive than the detection by UV absorbance, and with this sensitive and selective technique, 8OHdG has been identified and quantitated in DNA isolated from various sources including mouse liver [13], HeLa cells [13], rat kidney [6, 14], rat urine [6], and rat liver [15]. Recently, Ames and collaborators have used HPLC with electrochemical detection to assay 8OHdG in human urine [16, 17]. Their assay employed a series of solid phase extraction steps which separate 8OHdG from other urinary constituents, followed by analysis by gradient reversed phase HPLC coupled to a dual electrode high efficiency electrochemical detection system. Alternatively, we developed a sensitive assay for determination of 8OHdG in human urine based on coupled-column HPLC with electrochemical detection [18]. This assay utilises only one extraction step, which makes the entire system more easily automated, and thus enables the processing and analysis of a large number of samples within a limited amount of time. Here, we describe such an automated assay system for urinary 8OHdG with large sample capacity and illustrate how it can be applied for assessing *in vivo* oxidative DNA damage in cancer patients.

MATERIALS AND METHODS

Urine collection

Collections of urine every 24 h were obtained from 7 healthy individuals, 4 men (aged 40–45 years) and 3 women (aged 24–52 years), and from 30 cancer patients, 20 men (aged 19–74 years) and 10 women (aged 31–68 years). The diagnoses were breast cancer (n = 5), malignant teratoma (n = 5), gastric cancer (n = 4), head and neck cancer (n = 4), sarcoma (n = 3), malignant lymphoma (n = 3), brain tumour (n = 3), lung cancer (n = 1), pancreatic cancer (n = 1), and colorectal cancer (n = 1). Altogether, 27 urinary collections were obtained from the healthy individuals (15 from the men and 12 from the women) and 136 from the cancer patients. The urine was collected in ordinary plastic bottles without any additives and stored at −20°C until analysed.

Random urinary samples (2 × 10 ml) were obtained from 144 normal healthy subjects (74 men and 70 women) with ages ranging from 14 to 87 years, and from 141 patients suffering from malignant diseases: 89 with breast cancer, 14 with lung cancer, 13 with colonic cancer, 17 with malignant lymphoma, and 8 with malignant teratoma. The urine samples were collected and 2 × 10 mL aliquots from each sample were stored at −20°C until analysed.

Urinary samples were also obtained from 7 patients undergoing whole body irradiation therapy in conjunction with autologous bone marrow transplantation. The diagnoses in these cases were acute myeloid leukaemia (n = 3), acute lymphatic leukaemia (n = 3), and Hodgkin's disease (n = 1). The patients were given cyclophosphamide (60 mg/kg body weight) on the sixth and seventh days before transplantation, and received whole body irradiation (total dose 12 Gy) on four consecutive days before transplantation. Urine was collected at different time points during the treatment: before, during and after the irradiation.

The influence of certain specific cytostatic combinations on the urinary 8OHdG excretion was studied in 4 patients undergoing chemotherapy for different malignant diseases. 1 patient (male, 73 years old) received carboplatin and 5-fluorouracil (5-FU) for laryngeal carcinoma which had spread to the regional lymph nodes and the lungs. The second patient (female, 46 years old) received adjuvant therapy with doxorubicin and cisplatin after excision of mandibular osteosarcoma. The third patient (male, 68 years old) received etoposide, 5-fluorouracil and leucovorin for palliation of disseminated gastric cancer, and the fourth patient (male, 41 years old) was treated with cisplatin and 5-FU against a locally spread oral cancer. In all 4 cases, urine was collected from the onset of the therapy and during the first 2–6 days of treatment.

Chemicals and reagents

The 8OHdG was synthesised and handled and its concentration determined as described earlier [19]. All chemicals were of reagent grade quality and methanol was of HPLC quality. BondElut Cyclohexyl columns (Bondelut CH, 500 mg, 3 mL) were from Varian (Harbor City, California, U.S.A.).

Sample preparation

The pH of urine was adjusted to 6–7 by the addition of HCl or NaOH, 1 mol/L. The urine was then centrifuged and the clear supernatant used for analysis.

Urine samples were processed by a Gilson ASPEC sample processor (Gilson Medical Electronics, Middleton, Wisconsin, U.S.A.). The ASPEC was equipped with a 500 mg column kit and a Rheodyne injection valve. Sixty samples were loaded in one working session, which lasted for about 80 h. The samples were processed and then injected on line, one after the other. Injection was performed by overfilling the 100 μL Rheodyne loop with 600 μL of the sample. The working cycle for one sample is as follows: the Bondelut CH column was activated with 3 mL of methanol, followed by 3 mL of Tris buffer, 50 mmol/L, pH 7.0. Urine, 2.5 mL, was then applied and the column washed with 5 mL of Tris buffer, 50 mmol/L, pH 7.0. 8-Hydroxydeoxyguanosine was then eluted in a collection tube with 2.5 mL of Tris buffer containing 20% methanol (v/v). The eluate was mixed and 100 μL injected on line in the HPLC system.

HPLC equipment

The HPLC system configuration was essentially as described earlier [19] with the following exceptions: the manual injector was replaced by the Gilson sample processor described above. The column in dimension I was a 150 × 4.1 mm PRP-1 (Hamilton, Bonaduz, Switzerland), particle size 5 μm, operated at 40.0°C, and the column in dimension II, a 250 × 4.6 mm Apex ODS II (Jones Chromatography, Hengoed, Mid-Glamorgan, U.K.), particle size 5 μm, operated at 24.0°C. In dimension I, a PRP-1 precolumn (25 × 2.3 mm) from Hamilton was used. The eluate from dimension I was passed through a 2 mL loop immersed in water to bring the eluate back to room temperature. Pulse dampening was considered not necessary. The WAVS valve station was controlled by a ChromJet integrator (Spectra-Physics, San Jose, California, U.S.A.). Detection in dimension I was achieved with a TL-5A glassy carbon cell (Bioanalytical Systems, West Lafayette, Indiana, U.S.A.) operated at +600 mV versus a Ag/AgCl/3 M NaCl reference electrode. A

Zäta LC-4B (Zäta-Elektronik, Eslöv, Sweden) was used to operate the glassy carbon cell. Detection in dimension II was achieved with a Waters 460 electrochemical detector (Millipore, Milford, Massachusetts, U.S.A.) equipped with a glassy carbon cell operated at +600 mV versus the Ag/AgCl/3 M KCl reference electrode.

Analysis

The mobile phase in dimension I was phosphoric acid, 20 mmol/L, EDTA, 0.2 mmol/L, and heptanesulphonic acid sodium salt, 5 mmol/L, adjusted to pH 4.0 by the addition of sodium hydroxide, 5 mol/L. In dimension II, the mobile phase was a mixture of phosphate, 50 mmol/L, EDTA, 0.2 mmol/L, pH 7.0 and methanol 19 : 1 (v/v). The solutions were prepared in 5 L batches and used within 14 days. The flow was 0.75 mL/min in dimension I and 1 mL/min in dimension II. Washing solutions for the columns were prepared by addition of methanol to the solutions above to make a concentration of methanol of 50% (v/v). All these four solutions were filtered by vacuum through a 0.45 μm cellulose acetate filter.

At the beginning of every working session, a 1000 nmol/L standard was injected at least twice to check the retention time of the 8OHdG on the dimension I column. The valve after the column was therefore set to direct the flow through the TL-5A glassy carbon electrode. When the retention time had been determined, the valve was set to direct the flow from the dimension I column to the valve station. The valve station was then programmed to inject the 2 mL fraction from column I appearing up to 1 min after the retention time of 8OHdG on the dimension I column. To avoid the appearance of "ghost" peaks, it was found necessary to wash both columns after each run with the solutions with 50% methanol as mentioned above.

Standard curves were linear up to at least 500 nmol/L and calibration was carried out using a 32 nmol/L standard. A quality control programme was made up of three control samples (high, medium and low levels) analysed at the start, middle, and end of each series. These control samples were prepared by addition of 8OHdG to a 24 h urine collection from a healthy individual. Based on this quality control programme, no correction for drift in the method was found to be necessary.

Statistical determinations

Statistical testing of differences between means of different patient groups was made with the two-tailed Student's *t*-test.

RESULTS

Analysis of urinary 8OHdG

Under the conditions described, 8OHdG eluted as a single peak after 30 min and was separated from other peaks in the chromatogram (Figure 1). The lowest detectable concentration of 8OHdG was 0.6 nmol/L urine, calculated as twice the standard deviation of 40 duplicate samples containing less than 6 nmol/L. The coefficient of variation within the series (calculated from control samples run at each working session) was 8–24%, and between series 8–23% (Table 1), depending on the concentration level. The coefficient of variation within series calculated from analyses of duplicate samples was 4.9–6.8%.

Urinary 8OHdG excretion

The automated HPLC system was used to measure the urinary 8OHdG excretion in healthy individuals and different groups of cancer patients. Table 2 shows the excretion in 24 h collections from different subjects. Healthy individuals excreted 14.9 ± 7.8 nmoles 8OHdG per 24 h (mean ± standard deviation), which corresponded to 9.1 ± 3.7 nmol 8OHdG/L urine and 1.11 ± 0.62 μmol 8OHdG/mol creatinine (8OHdG excretion was given as μmol/mol creatinine so as to adjust for degree of urine dilution). There were no significant differences between men and women with regard to 8OHdG excretion (assessed as nmol/24 h or μmol/mol creatinine).

Table 2 also shows 8OHdG excretion in 24 h collections from a variety of cancer patients. Compared with healthy individuals, the excretion (in μmol/mol creatinine) was significantly higher in the whole group of cancer patients (2.13 ± 1.40 versus 1.11 ± 0.62, $P < 0.001$) as well as in several subgroups (e.g. breast cancer, malignant lymphoma, and head and neck cancer). In accordance, the excretion of 8OHdG in nmol per 24 h was significantly higher in patients with breast cancer and malignant lymphoma than in healthy individuals, and this was also the case when 8OHdG excretion was assessed as nmol 8OHdG/L 24 hour urine without adjustment for creatinine (Table 2). In other subgroups of patients, the 8OHdG excretion per 24 h was slightly higher than in healthy individuals, but the differences were not statistically significant.

Since many, but not all, cancer patients were undergoing (or had recently been subject to) radiotherapy and/or chemotherapy at the time of urinary sampling, we were able to compare samples taken before ($n = 20$) and after ($n = 116$) onset of oncological therapy. It then appeared that the highest levels were found in samples taken after therapy (2.18 ± 1.44 μmol/mol creatinine, $P < 0.001$ versus healthy individuals), but increased levels were also found in samples collected before onset of oncological therapy (1.84 ± 1.12 μmol/mol creatinine, $P < 0.01$ versus healthy individuals).

8OHdG in random urinary samples

Table 3 shows 8OHdG levels in random urinary samples from healthy individuals and cancer patients. There were no significant differences between healthy men and women with regard to 8OHdG levels, neither in nmol/L nor in μmol/mol creatinine. By contrast, the mean 8OHdG concentration in samples from cancer patients was significantly higher than in samples from healthy individuals. Thus, mean values of μmol 8OHdG/mol creatinine in samples from patients with breast cancer, lung cancer, colonic cancer, malignant lymphoma and malignant teratoma were all significantly higher than in samples from healthy individuals ($P < 0.001$). Significant differences were also found between cancer patients and healthy individuals in terms of nmol 8OHdG/L urine ($P < 0.05$). This difference was particularly clear for patients with malignant lymphoma and malignant teratoma ($P < 0.001$ in both cases), but was also significant for patients with breast cancer ($P < 0.05$). The differences between the groups are also illustrated in Figure 2, which shows the 8OHdG concentration (in nmol/L and μmol/mol creatinine) in each sample analysed.

Since many, but not all, random urinary samples were taken after the onset of oncological therapy, the 8OHdG levels in samples taken before and after therapy were compared. As shown in Table 4, the highest levels were found in samples taken after therapy ($P < 0.001$ versus healthy individuals regardless of whether 8OHdG concentration is in nmol/L or μmol/mol creatinine). However, significantly increased levels of 8OHdG (as μmol/mol creatinine) were also found in samples taken before onset of therapy (1.91 ± 0.96 μmol/mol creatinine in cancer patients versus 1.19 ± 0.48 in healthy individuals, $P < 0.001$), and such increased levels were found in several subgroups:

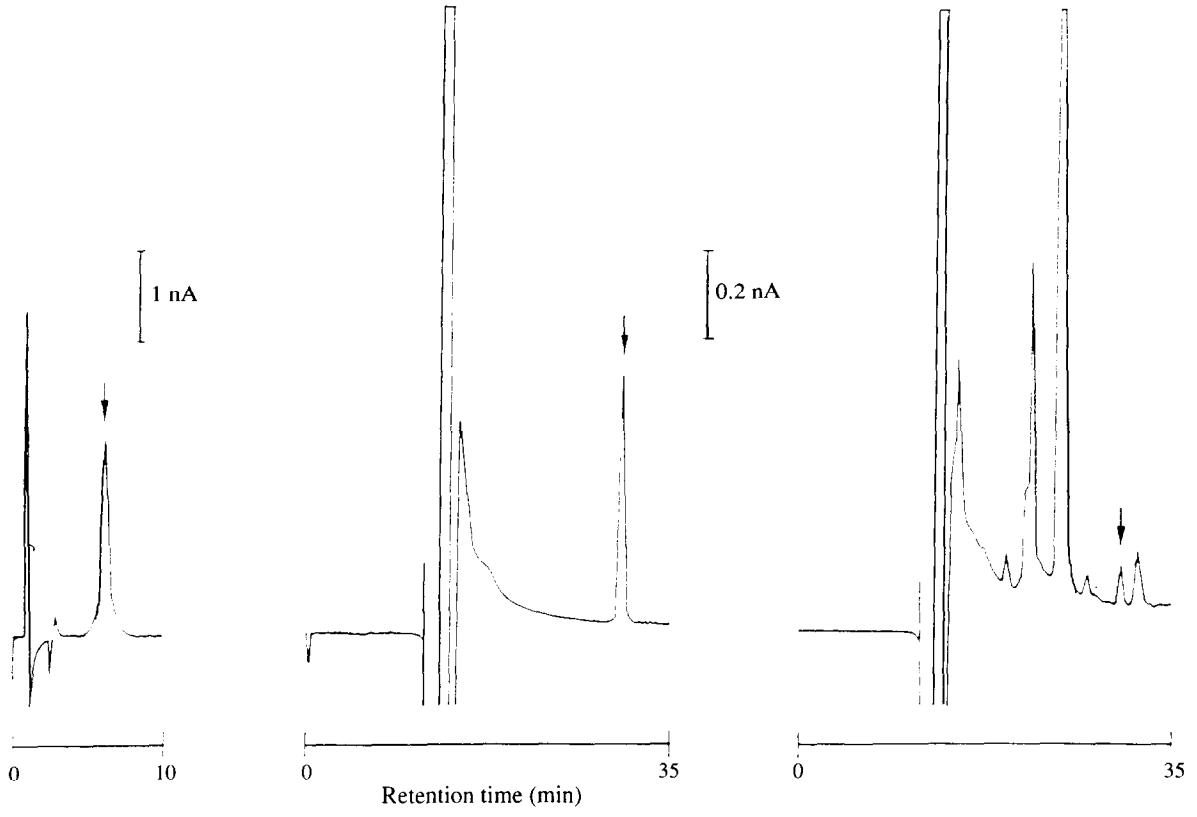

Figure 1. Coupled-column liquid chromatography of 8OHdG in normal human urine. Left: first dimension (620 nmol/L); middle: coupled system (32 nmol/L); right: coupled system (normal human urine). 8OHdG is indicated by arrow.

Table 1. Analytical precision for the automated analysis of urinary 8OHdG. Three different samples (containing "low", "medium" and "high" concentration of 8OHdG) were analysed at 15 different working sessions

	Urinary 8OHdG level		
	Low	Medium	High
Number of observations	45	44	43
Mean value*	7.43	15.7	31.1
Total variance	6.10	4.80	11.7
Within series variance	3.13	1.76	6.10
Within series CV (%)	24	8.4	7.9
Between series variance	2.97	3.04	5.6
Between series CV (%)	23	11	7.6

*nmol/L.

breast cancer ($P < 0.001$ versus healthy women), lung cancer ($P < 0.001$ versus healthy individuals), colonic cancer ($P < 0.05$ versus healthy individuals) and malignant lymphomas ($P < 0.001$ versus healthy individuals). Alternatively, levels of 8OHdG in nmol/L (i.e. without adjustment for urine dilution) were not significantly increased in samples taken before therapy (Table 4, first column). The differences between the groups of samples (i.e. from healthy individuals, cancer patients before therapy, and cancer patients after onset of therapy) are illustrated in Figure 3, which shows cumulative frequency distributions (%) of the 8OHdG concentrations in the different groups.

Influence of irradiation

The 8OHdG excretion in 7 patients subjected to whole body irradiation is illustrated in Figure 4. There was a marked increase in excretion following irradiation in 6 out of the 7 patients, and peak concentrations in the range of 35–55 μmol/mol creatinine were found in 3 cases. 1 patient, who had an aplastic bone marrow before the treatment, showed no increase in urinary 8OHdG excretion following irradiation (Figure 4).

Influence of cytostatic agents

The 8OHdG excretion in 4 patients receiving chemotherapy with different cytostatic agents is shown in Figure 5. There was a significant elevation in excretion following chemotherapy in 3 out of the 4 patients studied, with peak concentrations of 5–10 μmol 8OHdG/mol creatinine. 1 patient given carboplatin and 5-FU did not show any increase in 8OHdG excretion (Figure 5).

DISCUSSION

In this investigation, we demonstrated that 8OHdG in human urine can be analysed by automated coupled-column HPLC with electrochemical detection. Using this method, we found that normal healthy subjects excreted 14.9 ± 7.8 nmol 8OHdG per 24 h, or 1.11 ± 0.62 μmol 8OHdG per mol creatinine, in their urine. This corresponds to an average of 185 pmol per kilogram body weight per day (24 h), which is well in accordance with the value of Shigenaga and associates [16] who estimated the total amount of 8OHdG excreted by humans to be 130–300 pmol per kilogram body weight per day. In comparison, increased levels of 8OHdG were found in 24 h collections from

Table 2. Urinary 8OHdG excretion in 24 h collections from healthy individuals and cancer patients

Group	*n*	Urinary 8OHdG excretion nmol/L	nmol/24 h	μmol/mol creatinine
Healthy individuals	27	9.1 ± 3.7	14.9 ± 7.8	1.11 ± 0.62
Men	15	10.7 ± 4.0	16.4 ± 10.0	1.14 ± 0.84
Women	12	7.1 ± 2.2	13.0 ± 2.9	1.08 ± 0.15
Cancer patients	136	10.4 ± 5.6	18.0 ± 10.9	2.13 ± 1.40*
Breast cancer	22	11.4 ± 3.7†	20.2 ± 7.5‡	1.97 ± 0.87‡
Malignant lymphoma	13	14.7 ± 4.8*	21.6 ± 12.5§	1.82 ± 1.02¶
Malignant teratoma	13	12.1 ± 6.5	17.7 ± 11.1	1.39 ± 0.67
Head and neck	34	7.1 ± 4.0	17.2 ± 11.0	2.19 ± 1.56‖
Miscellaneous	54	10.6 ± 6.0	16.8 ± 11.5	2.41 ± 1.60*

* $P < 0.001$ versus healthy individuals; † $P < 0.001$ versus healthy women; ‡ $P < 0.01$ versus healthy women; § $P < 0.05$ versus healthy individuals; ¶ $P < 0.01$ versus healthy individuals; and ‖ $P < 0.05$ versus healthy men.
Values are means ± standard deviations; *n*, number of samples analysed.

Table 3. 8OHdG levels in random urinary samples from healthy individuals and cancer patients

Group	*n*	Urinary 8OHdG nmol/L	μmol/mol creatinine
Healthy individuals	129	14.6 ± 7.5	1.19 ± 0.48
Men	71	15.4 ± 7.4	1.13 ± 0.47
Women	58	13.7 ± 7.5	1.27 ± 0.49
Cancer patients	436	20.5 ± 19.3*	2.42 ± 2.28‡
Breast cancer	255	18.1 ± 19.2†	2.21 ± 1.40¶
Lung cancer	46	16.2 ± 7.0	2.11 ± 1.22‡
Colonic cancer	28	17.2 ± 10.6	1.93 ± 0.80‡
Malignant lymphoma	77	27.8 ± 32.0‡	3.05 ± 3.48‡
Malignant teratoma	30	32.0 ± 23.8§	3.95 ± 4.65§

* $P < 0.05$ versus healthy individuals; † $P < 0.05$ versus healthy women; ‡ $P < 0.001$ versus healthy individuals; § $P < 0.001$ versus healthy men; and ¶ $P < 0.001$ versus healthy women.
Values are means ± standard deviations; *n*, numbers of samples analysed.

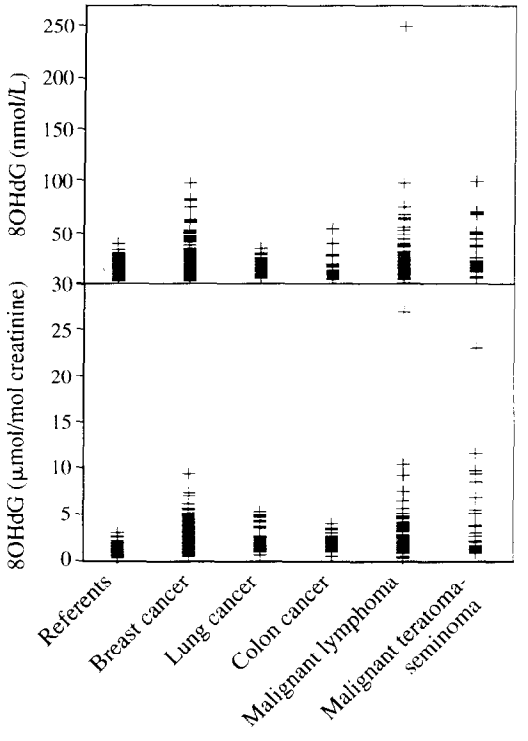

Figure 2. 8OHdG levels in random urinary samples from healthy individuals (referents) and different groups of cancer patients. Number of samples analysed were: referents, 129; breast cancer, 255; lung cancer, 46; colonic cancer, 28; malignant lymphoma, 77; and malignant teratoma, 30.

several groups of cancer patients (breast cancer, malignant lymphoma, and head and neck cancer). Furthermore, mean values of 8OHdG (μmol/mol creatinine) in random urinary samples from cancer patients (breast cancer, lung cancer, colonic cancer, malignant lymphoma, and malignant teratoma) were significantly higher than in random samples from healthy subjects (Table 3).

It is suggested that, as a group, patients with malignant diseases, may have increased urinary excretion of 8OHdG and by implication, increased oxidative DNA damage. However, many of the patients were undergoing (or had been subjected to) radiotherapy and/or chemotherapy at the time of urinary sampling, and it is therefore unclear whether the increased 8OHdG excretion was due to the disease or the therapy or both. An attempt was made to separate these factors by comparing samples taken before and after onset of oncological therapy. The results obtained indicate that the highest 8OHdG levels were found after therapy, but that mean 8OHdG levels (in μmol/mol creatinine) were also elevated in samples taken before therapy. Moreover, as illustrated in Figure 3, the group of samples taken before therapy in cancer patients were clearly different from the group of control samples. Although we can offer no conclusive explanation for this finding, the possibility is raised that cancer patients, on a group basis, have increased DNA damage as assessed by 8OHdG excretion. Alternatively, when 8OHdG excretion was assessed as nmol/L in random samples, there was no significant difference between cancer patients before therapy onset and healthy subjects, indicating that the discrimination was dependant on adjustment for urinary dilution. In samples from cancer patients taken after therapy onset, however, the 8OHdG levels were significantly increased, regardless of whether values were adjusted for creatinine or not.

It is reasonable to assume that the increased excretion in cancer patients after therapy onset was largely due to the therapy.

Table 4. 8OHdG levels in random urinary samples from cancer patients taken before and after onset of oncologic therapy

Group	Urinary 8OHdG nmol/L Before	nmol/L After	μmol/mol creatinine Before	μmol/mol creatinine After
Cancer patients (all)	15.8 ± 7.8(79)	21.5 ± 20.8(357)*	1.91 ± 0.96(79)*	2.57 ± 2.46(357)*
Breast cancer	15.1 ± 8.3(51)	18.8 ± 15.3(204)†	1.93 ± 0.97(51)‡	2.28 ± 1.48(204)‡
Lung cancer	15.2 ± 7.5(12)	16.5 ± 6.9(34)	1.96 ± 1.08(12)*	2.16 ± 1.28(34)*
Colonic cancer	18.0 ± 7.4(7)	16.9 ± 11.6(21)	1.57 ± 0.52(7)§	2.05 ± 0.86(221)*
Malignant lymphoma	21.1 ± 6.3(5)	28.2 ± 33.0(72)*	2.64 ± 1.15(5)*	3.08 ± 3.59(72)*
Malignant teratoma	15.4 ± 2.7(4)	34.5 ± 24.6(26)¶	1.25 ± 0.06(4)	4.36 ± 4.87(26)¶

* $P < 0.001$ versus healthy individuals; † $P < 0.05$ versus healthy women; ‡ $P < 0.001$ versus healthy women; § $P < 0.05$ versus healthy individuals; and ¶ $P < 0.001$ versus healthy men.
Values are means ± standard deviations, numbers of samples analysed are given in parentheses.

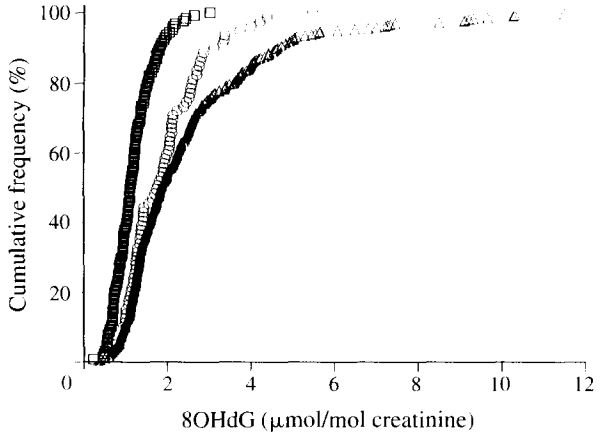

Figure 3. Cumulative frequency distributions (%) of 8OHdG concentrations (μmol/mol creatinine) in random urinary samples from healthy individuals (□), cancer patients before oncological therapy (○), and cancer patients after oncological therapy (△).

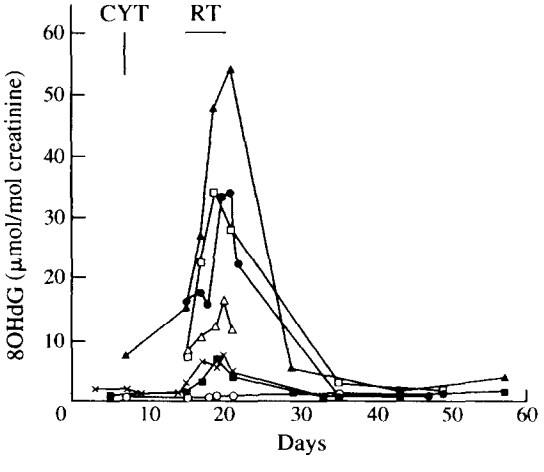

Figure 4. Urinary excretion of 8OHdG in patients subjected to whole body irradiation. The patients were given cyclophosphamide (60 mg/kg body weight) for 2 days (CYT), and whole body irradiation (total dose 12 Gy) for 4 days (RT) prior to autologous bone marrow transplantation. The diagnoses were acute myeloid leukaemia (□,●,○), acute lymphatic leukaemia (△,▲,■), and Hodgkin's disease (X). Each line represents 1 patient.

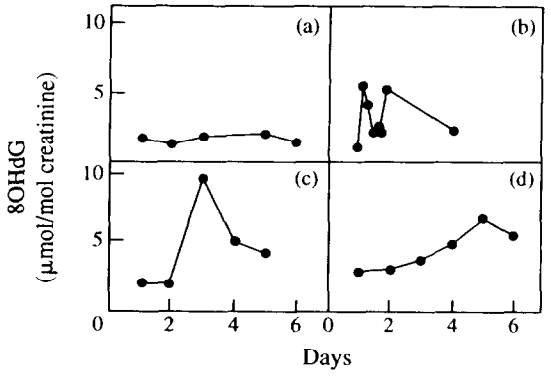

Figure 5. Urinary excretion of 8OHdG in patients receiving cytostatic agents. (a) Laryngeal cancer patient given carboplatin 400 mg/m² day 2 and 5-FU 1000 mg/m² days 2–6. (b) Osteosarcoma patient given cisplatin 60 mg/m² day 2 and doxorubicin 20 mg/m² days 2–4. (c) Gastric cancer patient given etoposide 120 mg/m² days 2–4 and 5-FU 500 mg/m² + leucovorin 300 mg/m² days 2–4. (d) Oral cancer patient given cisplatin 100 mg/m² day 2 and 5-FU 1000 mg/m² days 2–6.

As reported by Simic and coworkers [19], partial body irradiation of patients induces excretion of 8OHdG in the urine, and several anticancer drugs have been shown to induce generation of reactive oxygen species *in vitro* [20–24] and to cause free radical mediated cellular damage *in vivo* following chemotherapy in patients with cancer [25]. Clearly, much more investigation is needed to clarify the reasons why patients may have increased 8OHdG excretion. Nevertheless, the present findings demonstrate that human cancer patients undergoing radiotherapy and/or chemotherapy may show increased urinary 8OHdG excretion, and that this increase can be determined using automated coupled-column HPLC-EC as described in the present investigation. They also suggest that cancer patients not subjected to radiotherapy and/or chemotherapy may show increased urinary 8OHdG excretion, possibly due to increased oxidative stress and high rates of DNA damage and repair.

As illustrated in Figure 4, 6 of 7 patients treated with total body irradiation to a dose of 12 Gy showed increased levels of urinary 8OHdG, and 4 patients showed levels 8–25 times higher than in healthy individuals. This is in line with the anticipated effect of ionising irradiation on the DNA molecule, and may serve to illustrate the possible use of the technique for studying effects of radiotherapy at the molecular level. 1 patient, however, showed no increase. The reason for this is presently unknown.

The patient had an aplastic bone marrow, and it is possible that effects on the bone marrow are reflected in the urinary 8OHdG levels. This would be consistent with the notion that increased urinary 8OHdG may reflect the action of ionising irradiation on radiosensitive tissues.

As indicated in Figure 5, 3 of 4 patients undergoing chemotherapy with certain cytostatic drugs showed increased urinary 8OHdG excretion. This suggests that a number of cytostatic drugs are able to induce the formation of 8OHdG in the DNA molecule, although the mechanism(s) by which this is achieved is as yet unclear. One possibility is that many cytostatic drugs in fact increase the degree of oxidative stress in cells, thereby promoting the formation of hydroxyl radicals that may attack carbon 8 on the deoxyguanosine residue. However, the results of the present investigation are based on few observations, and further studies are required to clarify the relation between cytostatic drug therapy and urinary 8OHdG excretion. Among the 4 patients studied (cf. Figure 5), there seemed to be a correlation between the severity of immediate toxic effects from the treatment on one hand, and the level of urinary 8OHdG on the other. Thus, the patient with no increase in urinary 8OHdG excretion due to cytostatic treatment (Figure 5a) accepted the treatment best and showed few toxic symptoms. This indicates that increased urinary 8OHdG after cytostatic drug treatment may reflect increased cell death and ensuing DNA turnover. Dead, disrupted cells are known to undergo lipid peroxidation faster than healthy cells [26]: perhaps they also undergo oxidative DNA damage faster so that excretion of DNA base damage products is not necessarily a reflection of the extent of oxidative DNA damage in healthy cells. This area remains to be explored.

We have thus found that 8OHdG in human urine can be determined by automated HPLC, and that this can be used to assess oxidative DNA damage in cancer patients in a relatively simple way. Although the method should be further improved, particularly for measuring low levels of urinary 8OHdg in a precise way, it might prove useful clinically to investigate the increased 8OHdG levels associated with radio- and chemotherapies. It is thus possible that the responders to a particular therapy produce more 8OHdG than the non-responders. Finally, it should be emphasised that the results of the present investigation are based on unselected clinical materials, and that no attempts have been made to analyse the influences of age, disease activity at the time of the investigation, course of the disease in the various cases, type of treatment in each specific patient etc. This is because the aim of this first investigation was to illustrate the power of the analytical technique rather than to conduct carefully planned studies in homogeneous clinical materials. Further studies will have to take these considerations in account and focus on specific issues in selected groups of cancer patients.

1. Ames BN. Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. *Science* 1983, **221**, 1256–1264.
2. Cerutti PA. Prooxidant states and tumor promotion. Evidence for a role of prooxidant states in carcinogenesis. *Science* 1985, **227**, 375–380.
3. Troll W, Wiesner R. The role of oxygen radicals as a possible mechanism of tumor promotion. *Ann Rev Pharmacol Toxicol* 1985, **25**, 509–528.
4. Totter JR. Spontaneous cancer and its possible relationship to oxygen metabolism. *Proc Natl Acad Sci USA* 1980, **77**, 1763–1767.
5. Adelman R, Saul RL, Ames BN. Oxidative damage to DNA: relation to species metabolic rate and life span. *Proc Natl Acad Sci USA* 1988, **85**, 2706–2708.
6. Fraga CG, Shigenaga MK, Park J-W, Degan P, Ames BN. Oxidative damage to DNA during aging: 8-Hydroxy-2′-deoxyguanosine in rat organ DNA and urine. *Proc Natl Acad Sci USA* 1990, **87**, 4533–4537.
7. Dizdaroglu M, Bergtold DS. Characterization of free radical-induced base damage in DNA at biologically relevant levels. *Anal Biochem* 1986, **156**, 182–188.
8. Leadon SA, Hanawalt PC. Monoclonal antibody to DNA containing thymine glycol. *Mutation Res* 1983, **112**, 191–200.
9. Degan P, Shigenaga MK, Park E-M, Alperin PE, Ames BN. Immunoaffinity isolation of urinary 8-hydroxy-2′-deoxyguanosine and 8-hydroxyguanine and quantitation of 8-hydroxy-2′-deoxyguanosine in DNA by polyclonal antibodies. *Carcinogenesis* 1991, **12**, 865–871.
10. Cathcart R, Schwiers E, Saul RL, Ames BN. Thymine glycol and thymidine glycol in human and rat urine: A possible assay for oxidative DNA damage. *Proc Natl Acad Sci USA* 1984, **81**, 5633–5637.
11. Kasai H, Nishimura S. Hydroxylation of deoxyguanosine at the C-8 position by polyphenols and aminophenols in the presence of hydrogen peroxide and ferric ion. *Gann* 1984, **75**, 565–566.
12. Floyd RA, Watson JJ, Wong PK, Altmiller DH, Rickard RC. Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. *Free Rad Res Comms* 1986, **1**, 163–172.
13. Kasai H, Crain PF, Kuchino Y, Nishimura S, Outsuyama A, Tanooka H. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* 1986, **7**, 1849–1851.
14. Kasai H, Nishimura S, Kurokawa Y, Hayashi Y. Oral administration of the renal carcinogen, potassium bromate, specifically produces 8-hydroxydeoxyguanosine in rat target organ DNA. *Carcinogenesis* 1987, **8**, 1959–1961.
15. Richter C, Park J-W, Ames BN. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Natl Acad Sci USA* 1988, **85**, 6465–6467.
16. Shigenaga MK, Gimeno CJ, Ames BN. Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. *Proc Natl Acad Sci USA* 1989, **86**, 9697–9701.
17. Shigenaga MK, Ames BN. Assays for 8-hydroxy-2′-deoxyguanosine: A biomarker of *in vivo* oxidative DNA damage. *Free Rad Biol Med* 1991, **10**, 211–216.
18. Tagesson C, Källberg M, Leanderson P. Determination of urinary 8-hydroxydeoxyguanosine by coupled-column high performance liquid chromatography with electrochemical detection: a non-invasive assay for *in vivo* oxidative DNA damage in humans. *Toxicol Methods* 1991, **1**, 242–251.
19. Simic MG, Bergtold DS, Karam LR. Generation of oxy radicals in biosystems. *Mutation Res* 1989, **214**, 3–12.
20. Mimnaugh EG, Kennedy KA, Trush MA, Sinha BK. Adriamycin-enhanced membrane lipid peroxidation in isolated rat nuclei. *Cancer Res* 1988, **45**, 3296–3304.
21. Mimnaugh EG, Trush MA, Bhatnagar M, Gram TE. Enhancement of reactive oxygen-dependent mitochondrial membrane lipid peroxidation by the anticancer drug adriamycin. *Biochem Pharmacol* 1985, **34**, 848–856.
22. Rajagopalan S, Politi PM, Sinha BK, Myers CE. Adriamycin-induced free radical formation in the perfused rat heart. Implications for cardiotoxicity. *Cancer Res* 1988, **48**, 4766–4769.
23. Pritsos CA, Sartorelli AC. Generation of reactive oxygen radical through bioactivation of mitomycin antibiotics. *Cancer Res* 1986, **46**, 3528–3532.
24. Lown JW, Joshna AV, Chan HH. Reactive oxygen species leading to lipid peroxidation and DNA lesions implicated in the cytotoxic action of certain antitumour antibiotics. In Mc Briens DCH, Slatter TF, eds. *Free Radicals, Lipid Peroxidation and Cancer*. New York, Academic Press, 1982, 305–328.
25. Sangeetha P, Das UN, Koratkar R, Suryaprabha P. Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. *Free Rad Biol Med* 1990, **8**, 15–19.
26. Halliwell B, Gutteridge JMC. Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. *Lancet* 1984, **i**, 1396–1398.

Acknowledgements——Supported by grants from the Swedish Work Environment Fund, the Swedish Medical Research Council, Östgötaregionens Cancerfond, and Landstinget i Östergötland.