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Cancer Epidemiology, Biomarkers & Prevention

Research Article

Urinary 8-Oxoguanine as a Predictor of Survival in Patients Undergoing Radiotherapy

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Abstract

Background: Because of the importance to identify prognostic indicator for radiotherapy, herein we decided to check whether the parameters which describe oxidative stress/DNA damage may be used as a marker of the therapy. The aim of this work was to investigate whether fractionated radiotherapy of patients with cancer ($n = 99$) is responsible for oxidative DNA damage on the level of the whole organism and whether the biomarkers of the damage such as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) and its modified base 8-oxo-7,8-dihydroguanine (8-oxo-Gua) in urine and DNA may be used as a predictor of radiotherapy success.

Methods: All the aforementioned modifications were analyzed using techniques which involve high-performance liquid chromatography/electrochemical detection (HPLC/EC) or HPLC/gas chromatography-mass spectroscopy (GC-MS).

Results: Of all analyzed parameters only patients with significantly elevated urinary excretion of the 8-oxo-Gua with concomitant unchanged level of 8-oxo-dG in leukocytes DNA in the samples collected 24 hours after the first fraction in comparison to the initial level have significantly increased survival time (60 months after the treatment, survival of 50% of the patients who fulfill the above mentioned criteria, in comparison with 10% of the patients who did not).

Conclusions: Results of our work suggest that patients with higher urinary 8-oxo-Gua and concomitant stable level of 8-oxo-dG in leukocytes DNA, after 24 hours of the first dose should be regarded as better responder to radiotherapy as being at lower risk of mortality.

Impact: The above mentioned statement could make it possible to use these parameters as markers to predict the clinical success. *Cancer Epidemiol Biomarkers Prev; 21(4); 629–34. ©2012 AACR.*

Introduction

The interaction of ionizing irradiation with water is responsible for reactive oxygen species production and interaction of reactive oxygen species with cellular components may result in damage to biomolecules including DNA. This in turn may be responsible for cancer cell death. Therefore, it is possible that oxidative DNA damage which arises as a result of radiotherapy may be involved in the therapeutic effect of the ionizing irradiation (1).

However, one of a side effect of radiotherapy are radiation-induced second malignancies, which may arise as an effect of ionizing irradiation interaction with noncancerous tissues and may be of some concern especially in patient populations undergoing multiple rounds of radiation therapy and enjoying long survival (2). Because oxidative DNA damage has mutagenic and carcinogenic potential measurement of typical markers of the damage, like 8-oxo-7,8-dihydroguanine (8-oxo-Gua; ref. 3), in patients with cancer undergoing radiotherapy may be of great interest. It may be used to optimize condition of irradiation and reduce the risk of secondary cancers which is unavoidably associated with radiotherapy and may have an impact on survival time after the treatment.

Despite several factors with prognostic value which are available for conventional cancer therapy, there is no test of outcome of radiotherapy for individual prediction (none of them can predict the clinical success). Usually certain dose level is prescribed for a whole group of patients with cancer with similar diagnosis. This dose is set empirically taking into consideration the most sensitive patients. However, development of prognostic test would allow to predict in advance which patients may be treated with even the higher dose while in the other case to find another more successful modality.

The result of a previous article (4) suggest that urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) may be used as a predictor for individual radiosensitivity. The authors observed an increase of this modification in patients with breast cancer who received radiotherapy

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as adjuvant treatment after surgery. However, measurement of exclusively urinary excretion of the repair product(s) may be sometimes misleading because it gives no information about the oxidative steady state (rate of damage vs. repair) within cellular DNA and informs only about an average value of damage repair occurring sometimes in the past. Moreover, the presence of 2′-deoxyribonucleoside lesions in extracellular matrices is poorly defined, as there are no reports of a single DNA repair enzyme whose activity yields 8-oxo-dG whereas urinary 8-oxo-Gua measurements may be attributed entirely to DNA repair, mainly to OGG1 (8-oxo-7,8-dihydroguanine glycosylase) activity (5). Therefore, in our study several parameters which describe oxidative DNA damage were analyzed. In addition to urinary 8-oxo-dG we also determined the level of modified base (8-oxo-Gua) in urine as well as the background level of the modification in cellular DNA. The aim of this work was to investigate:

i whether fractionated radiotherapy of patients with head and neck cancer is responsible for oxidative DNA damage on the level of the whole organism and
ii whether the biomarkers of the damage such as urinary 8-oxo-dG or 8-oxo-Gua as well as the level of oxidative DNA damage in leukocytes (all of the parameters reflect oxidative DNA damage on the level of the whole organism) may be used as a predictor of radiotherapy success. Urinary excretion of 8-oxo-dG and 8-oxo-Gua was analyzed using techniques which involves high-performance liquid chromatography/electrochemical detection (HPLC/EC) or HPLC prepurification followed by gas chromatography with isotope dilution MS detection (HPLC/gas chromatography mass spectroscopy (GC-MS; ref. 6). In addition to unequivocal identification of the analyzed compounds and high sensitivity, the use of isotopically labeled internal standards compensates for potential losses of the analyses during sample work-up.

Materials and Methods

Patients

Analysis of daily excretion of 8-oxo-Gua and 8-oxo-dG with urine was done in a study group consisted of 99 patients with malignant cancer (III and IV degree of clinical stage). Leukocytes from peripheral blood samples for analysis of 8-oxo-dG level in cellular DNA were obtained from 71 patients from among the study group. The patients suffered from various malignant tumors, that is, head and neck cancer ($n = 42$), breast cancer ($n = 32$), lung cancer ($n = 11$), and prostatic cancer ($n = 14$).

All the patients were eligible for radiotherapy. Patients were treated by radical radiotherapy with 6-MeV photons. The Planning Target Volume (PTV) encompassed in all patients the primary tumor site and a margin of approximately 1.5 cm. Table 1 presents patients clinical characteristics.

Expression of the urinary excretion rates in nmol/L/kg/24 h can deliver more information, for example, enables measurement of the number of the repaired lesions per day per cell (7). However, in the present study urine were collected as spot samples and the concentrations of 8-oxo-dG and 8-oxo-Gua was adjusted by the creatinine concentration. Spot morning urine samples can be used to determine excretion rate assuming that creatinine excretion is unchanged like in our work (i.e., in a clinically controlled trial). Moreover, it was found that there is the statistically significant correlation of the urinary excretion between morning spot urine samples corrected for creatinine excretion and 24-hour urine samples (8).

Spot urine samples and blood were taken before the treatment and 1 day after the first fraction of irradiation. The patients were asked to abstain from vitamin supplementation for at least 1 month before the radiotherapy started and during the course of the treatment. Only these patients qualified.

The study was approved by the medical ethics committee of The Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland, no 88/1999; 141/2001; 189/2001; 241/2002 (in accordance with Good Clinical Practice, 1998), and all the patients gave informed consent.

Isolation of leukocytes from venous blood

Venous blood samples (18 mL) from the patients were collected. The blood was carefully applied on top of Histopaque 1119 solution (Sigma-Aldrich Inc.), and leukocytes were isolated by centrifugation according to the procedure laid down by the manufacturer.

Urine analysis

Urine sample preparation, HPLC purification, and GC-MS analysis were conducted as described earlier (9).

DNA isolation and 8-oxo-dG determination in DNA isolates

DNA from leukocytes was isolated using the method as described earlier (9). Determination of 8-oxo-dG by the mean of HPLC/EC technique was described previously (9, 10).

Statistical analysis

All results are expressed as means by the StatSoft, Inc. (2009). STATISTICA (data analysis software system), version 9.0. (www.statsoft.com; ref. 11; lic. no: JXVP002-E256522AR-E) was used for the statistical analysis.

The Student *t* tests (for variables with normal distribution—levels of oxidatively damaged DNA before and after radiotherapy) were carried out. For normal distribution, variables were analyzed by the Kolmogorov–Smirnov test with the Lilliefors correction.

The association between overall survival and the urinary excretion of 8-oxo-dG, 8-oxo-Gua, as well as the level of oxidative damage DNA in leukocytes was estimated using the method of Kaplan and Meier and assessed using

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Table 1. Patient treatment and follow-up characteristics

Patient characteristic	No. of patients (%)		
Total	99		
Age, y			
Median	59		
Range	28–81		
Sex			
Male	66 (66)		
Female	33 (33)		
ECOG performance status			
0–1	82 (89)		
≥2	17 (11)		
	Stage	Radiotherapy (total dose Gy)	Median survival time, mo
Head and neck cancer ($n = 42$)	III	66–70	12.3
Breast cancer ($n = 20$)	IIIa	45	11.6
Breast cancer ($n = 12$)	IIIb	45	10.8
Lung cancer ($n = 7$)	IIIb	46–48	8.3
Lung cancer ($n = 4$)	IV	46–48	7.4
Prostatic cancer ($n = 14$)	IIIa	68–72	16.1

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

the log-rank test. Statistical significance was considered at $P < 0.05$.

Results

For whole patient population the median values of 8-oxo-Gua in urine taken before treatment (sample I) was 14.84 (nmol/L/mmol/L creatinine; interquartile range, 9.26–21.20). After the first fraction of irradiation (sample II), the level decreased to the value of 12.71 (nmol/L/mmol/L creatinine; interquartile range, 8.76–18.72). This difference was statistically significant ($P = 0.0018$; Table 2).

However, for the distinct subpopulation of 32 patients the level of urinary 8-oxo-Gua increased significantly ($P = 0.0003$) from 9.60 (7.20–12.99) to 12.68 (9.65–20.34; Table 2).

There have been no significant differences between samples I and II for urinary excretion of 8-oxo-dG [the respective values were 3.12 (nmol/L/mmol/L creatinine; interquartile range, 2.29–4.41 and 3.02 (nmol/L/mmol/L creatinine; interquartile range, 2.09–4.74)] and for 8-oxo-dG in DNA isolated from leukocytes with medians reaching the values 6.58 of 8-oxo-dG per 10^6 dG (3.45–14.38) and 5.13 (3.62–13.82), respectively (Table 2).

A comparative analysis of total survivals with respect to possible differences between sample I and II was conducted for all these parameters.

Significant increase of the range of total survival (Fig. 1; median, 17.5 months) was found in the group of patients with increased level of 8-oxo-Gua in urine 24 hours after the first fraction of radiotherapy in comparison with patients with decreased level of this marker after treatment, median, 6.2 months ($P = 0.006$; log-rank test).

No significant differences has been noticed in the range of total survival concerning urinary 8-oxo-dG and 8-oxo-dG in cellular DNA (Table 2).

Table 2. The level of 8-oxo-Gua, 8-oxo-dG in the urine, and 8-oxo-dG in the leukocytes' DNA median (interquartile range)

	Before radiotherapy	After radiotherapy	*P*
	All patients		
Urinary 8-oxo-Gua (nmol/L/mmol/L creatinine)	14.84 (9.26–21.20)	12.71 (8.76–18.72)	0.0018
Urinary 8-oxo-dG (nmol/L/mmol/L creatinine)	3.12 (2.29–4.41)	3.02 (2.09–4.74)	0.69
Leukocytes' 8-oxo-dG/10^6 dG	6.58 (3.45–14.38)	5.13 (3.62–13.82)	0.13
	Subgroup patients with increase urinary 8-oxo-Gua ($n = 32$)		
Urinary 8-oxo-Gua (nmol/L/mmol/L creatinine)	9.60 (7.20–12.99)	12.68 (9.65–20.34)	**0.0003**

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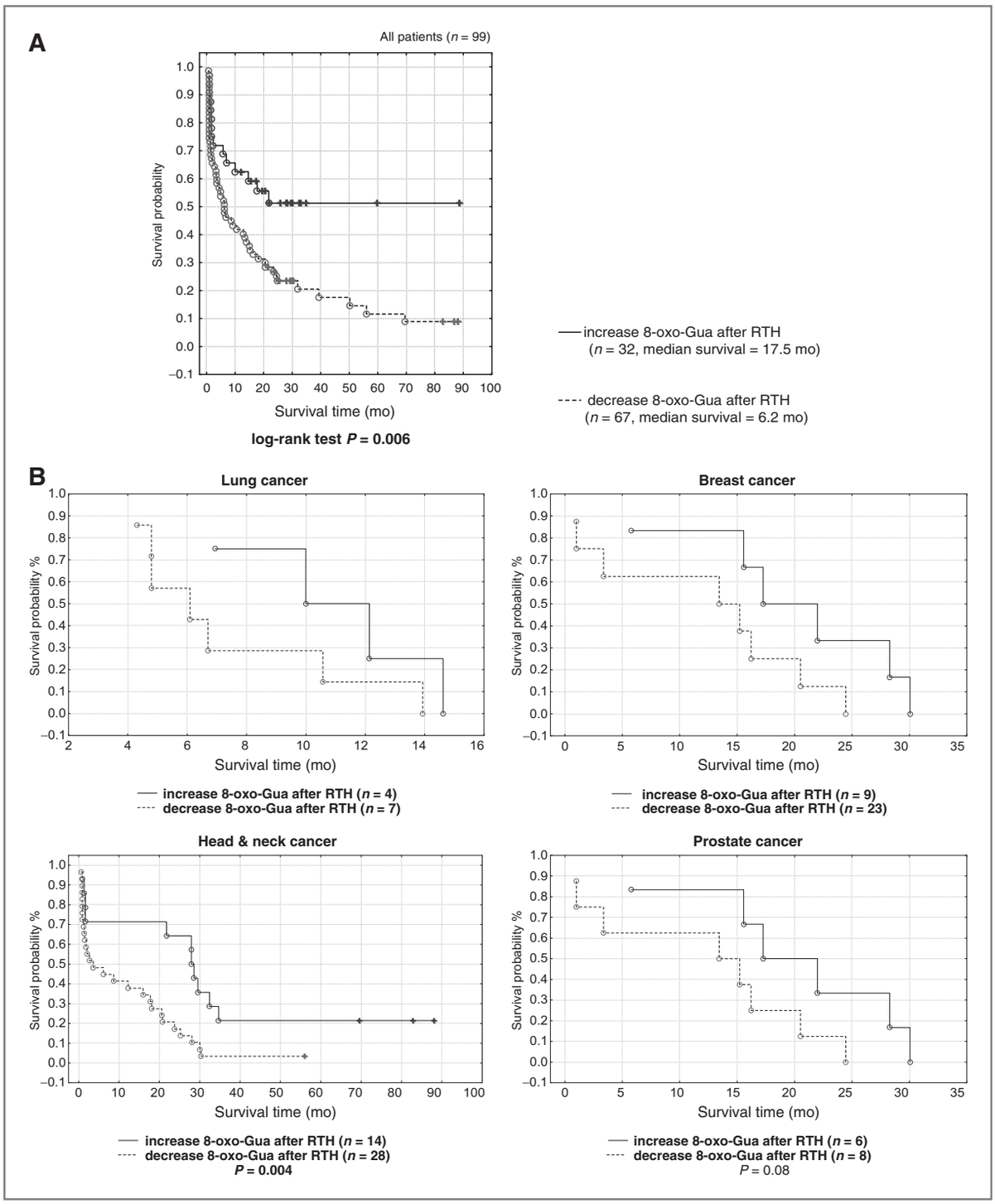

Figure 1. A, Kaplan–Meier curves of 8-oxo-Gua in urine after the first fraction of radiotherapy (RTH, all patients). B, Kaplan–Meier curves of 8-oxo-Gua in urine after the first fraction of radiotherapy (lung, breast, prostate, and head and neck cancers).

There were no differences among the patients with various stages of the disease development concerning all of the analyzed modifications (data not shown). No significant differences were found among the studied time points with respect to creatinine clearance and creatinine concentration (data not shown). A similar observation was reported by others (12).

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Discussion

In our previous article (13) with small group of patients ($n = 27$) undergoing fractionated radiotherapy, we have analyzed urinary excretion rates of 8-oxo-Gua and 8-oxo-dG as well as 8-oxo-dG in cellular DNA in the samples collected 24 hours after the last fraction (7 weeks after beginning of the therapy). It has been concluded that in the case of some patients with the lowest activity of OGG1, the combination of reduced OGG and irradiation was associated with increased background level of 8-oxo-Gua in cellular DNA. However, the assumption of the predictive test is to have some insight on results at the beginning of the therapy. Individuals vary in their radiation sensitivity and this variable response is a major problem in radiotherapy as radiation sensitivity or resistance of individuals can not be reliably predicted before the therapy. Therefore, it is a great need to elucidate the mechanism(s) responsible for enhanced or reduced sensitivity to radiotherapy and to develop suitable assay for a clinical use based on results as early of the treatment as possible. It is also important to have significant follow-up period for evaluation of the treatment effect. Therefore, herein we have analyzed all the parameters which describe oxidatively damaged DNA 24 hours after the first fraction and have evaluated survival time of 99 patients during 60-month follow-up period.

Of all analyzed parameters only patients with significantly elevated urinary excretion of the modified base with concomitant unchanged level of 8-oxo-dG in leukocytes' DNA in the samples collected 24 hours after the first fraction in comparison to the initial level have significantly increase survival time (60 months after the treatment, survival of 50% of the patients who fulfill the above mentioned criteria, in comparison with 10% of the patients who did not). It should be remembered that the level of the modified base in urine may be an indicator of oxidative insult to DNA, a general marker of oxidative stress, or perhaps reflective of DNA repair, considering stable level of the modification in cellular DNA (reviewed in 4).

An important question is why these parameters are meaningful to predict longer survival of the patients undergoing radiotherapy?

In the both patients groups no changes in background level of 8-oxo-dG in DNA was observed. Therefore, increase in urinary excretion of the modified base in the group with longer life expectancy is, most likely, a measure of better response to the radiation (higher radiosensitivity) and simultaneously efficient removal of the damage from cellular DNA of this group (14). Because 8-oxo-Gua is a repair product of the DNA damage, it is likely that, at least in the case of some patients with the higher activity of OGG1 the combination of higher OGG1 repair capacity and irradiation was associated with stable background level of 8-oxo-Gua in cellular DNA. Apparently higher efficiency of DNA repair is able to cope with the radiation induced, the extra amount of 8-oxo-Gua leading to a reduction of potentially mutagenic/carcinogenic lesions.

There are individual differences with respect to the formation as well as the removal of the modifications after radiotherapy. Therefore, the possibility exists that this variability may partially account for the differences in clinical response to the therapy. This individual variability may reflect individual differences in metabolism and repair capacity and, at least in part, genetic background (15, 16).

As mentioned earlier, urinary 8-oxo-Gua is a marker of oxidative insult to DNA and a general marker of oxidative stress. Because in the good prognosis group there was the lower preradiation 8-oxo-Gua level than in the whole population (see Table 2), it is a possibility that lower initial oxidative stress/DNA damage is one of a parameter which may influence longer survival time.

In conclusion, because of the importance to identify prognostic indicator for radiotherapy, herein we decided to check whether the parameters which describe oxidative stress/DNA damage may be used as a marker to predict a success of the therapy. Results of our work suggest that patients with higher urinary 8-oxo-Gua and concomitant stable level of 8-oxo-dG in leukocytes DNA, after 24 hours of the first dose should be regarded as better responder to radiotherapy as being at lower risk of mortality. This statement could make it possible to use these parameters as markers to predict the clinical success.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K. Roszkowski, R. Olinski
Development of methodology: K. Roszkowski
Acquisition of data (acquired and managed patients, provided facilities, etc.): K. Roszkowski, R. Olinski
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, and computational analysis): K. Roszkowski, R. Olinski
Writing, review, and/or revision of the manuscript: K. Roszkowski, R. Olinski
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Roszkowski
Study supervision: K. Roszkowski

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