In: Kumpulainen JT & Salonen JT eds. Natural antioxidants and food quality in atherosclerosis and cancer prevention. The Royal Society of Chemistry 1996: 78-82.

MEASUREMENT OF OXIDATIVE DNA INJURY IN HUMANS: EVALUATION OF A COMMERCIALLY AVAILABLE ELISA ASSAY

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1 INTRODUCTION

DNA modification can originate from reaction between DNA and a variety of chemicals, e.g. aromatic carcinogens. Most often the reaction is not between DNA and the chemicals per se but metabolic products which are more reactive. The DNA modifying agents of interest now expand to oxygen. Recently a variety of oxidative modifications of DNA have been demonstrated[1-6] and it appears that their occurrence is more frequent than modifications from other chemicals. Furthermore, it has now been demonstrated that modification from reactive oxygen species (ROS) can result in the same mutations as resulting from aromatic carcinogens, e.g. G-T transversion mutation in tumor suppressor gene p53 and codon 12 activation of c-Ha-ras or K-ras oncogenes[7-10].

The oxidative modifications have been estimated to approximately 10^4-10^5 base modifications per cell per day[11]. If unrepaired this would mean almost total oxidation of all DNA bases in the human maximum lifespan of about 100 years. It is therefore, and from a long list of other observations reasonable to assume a very efficient and almost complete repair of oxidative DNA modifications. Unrepaired oxidative modifications are probably few, but the rate at which the oxidation occurs is presumably predictive for the risk of mutation whereas the tissue levels of oxidized bases are determined by the balance between oxidation and repair.

Oxidation of the guanine base is the most prominent and mutagenic oxidative modification and its repair results in urinary excretion of the oxidatively modified base 8-oxoguanine (8-oxoGua) and the nucleoside 8-oxodeoxyguanosine (8-oxodG), the latter presently the most promising candidate as a biomarker of the rate of DNA oxidation also for estimation in specific tissues[12].

The assay possibilities for 8-oxodG in urine or tissue range from advanced HPLC-MS, GC-MS and multidimentional HPLC-EC to commercially available ELISA assay. In general the urine analysis presents more methodological problems than tissue analysis. We compared a commercial ELISA method to the HPLC-EC method on urine samples.

2 MATERIALS AND METHODS

Urine samples were collected as part of a large ongoing randomized smoking cessation study. From this study three healthy subjects were selected on the basis of high, intermediate and low urine concentrations of 8-oxodG estimated by HPLC. As part of the study the volunteers gave urine samples before entering the smoking cessation programme and after four and 26 weeks.

2.1 HPLC Assay to Measure 8-oxodG

As described in details elsewhere urine samples mixed with TRIS-HCl pH 2.9 are injected onto a 3 column HPLC system with computerized control and integration[13]. On the first column urine is chromatographed at pH 7.9 and from this "extraction column" a fast operating switching valve directs a small fraction containing the 8-oxodG to be trapped onto a small cation exchange column. From this column the fraction is eluted at pH 2.8 onto a high resolution C18 column for quantification by electrochemical detection from individual standards. Samples were analysed in duplicates with an intra and inter assay coefficient of variation of 10% and 13%, respectively. For analysis of the standards (see below) a single column HPLC assay was used[12].

2.2 ELISA Assay to Measure 8-oxodG

A commercial assay from GEN:OX was used (Genox Corporation, Baltimore, US). The kit includes 8-oxodG standards, reagents and a 96 well microtiter plate precoated with 8-oxodG. The procedure follows conventional ELISA methodology with application of 50 µl of sample or standard. After reaction the plate is read at 492 nm. The primary antibody is monoclonal, the secondary is horse radish peroxidase-conjugated antimouse polyclonal antibody. Lyophilized urine samples (5x concentration) were also prepared but the readings were optimal using untreated urine. The coefficient of variation for duplicate samples was 9.9% (n=12). Uric acid [1, 4, 10 mM], guanine, guanosine, deoxyguanosine, deoxyadenosine, and 8-oxoGua [2.3, 11.3, 546.5, 283 and 1413 nM] were tested in the ELISA assay but did not give any reaction.

2.3 Standards

The HPLC assay was calibrated by 8-oxodG samples with close to 100% purity kindly donated by Dr. Per Leanderson, Department of Occupational Medicine, University of Linköping, Sweden and by Dr. David W. Potter, Rohm and Haas Co., Spring House, P.A., US. The two standards deviated by a few per cent only. Standards from the ELISA kit and the former mentioned standards differed in the HPLC assay with at factor 2.13 (ELISA standard vs HPLC standard) evaluated by the slopes of standard curves up to 400 nM.

3 RESULT

The mean 8-oxodG concentration estimated by HPLC was 15.0 nM (sd=10.2) which was significantly different from the ELISA mean concentration of 48.6 nM (sd=39.0), p=0.008 by paired t-test. The ratios of ELISA to HPLC concentrations ranged 3 to 30, with a mean of 8.3 and sd=7.4, taking into account the difference in standards used for the two assays.

The actual concentrations of 8-oxodG estimated by ELISA and HPLC without correcting for difference in standards are depicted in Figure 1. The correlation between the ELISA and the HPLC estimate was 0.42 (r) and regression analysis gave a slope of 1.60 and an intercept of 24.39 nM.

The urinary 24 hour 8-oxodG excretion before and at 4 and 26 weeks after smoking cessation is depicted in Figure 2. By HPLC estimation there is a tendency to lowered 8-oxodG excretion after smoking cessation. By ELISA the values are considerably higher and appear more variable.

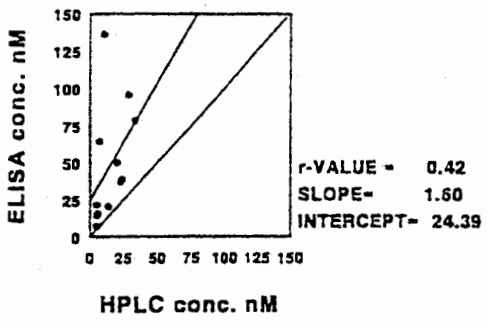

Figure 1
Correlation between concentration of 8-oxodG determined by HPLC and ELISA technique in 4 subjects at 3 occasions

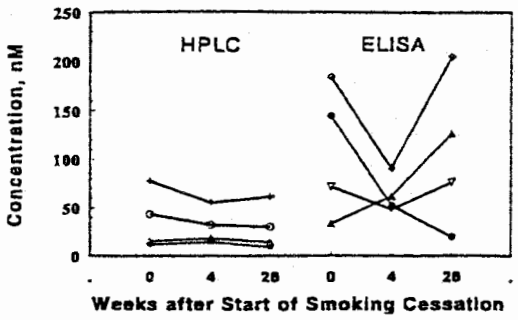

Figure 2
Urinary excretion of 8-oxodG in 4 smokers following smoking cessation measured by HPLC and ELISA technique

Table 1 *Published Values Regarding Urinary Biomarkers of Oxidative DNA-damage in Humans*

Experimental Protocol	*Age*	*Lesion*	*Assay*	*Excretion (mean_SD or range) per 24 hours*	*Ref.*
5 healthy subjects	unknown	8-oxodG	HPLC	323±23 pmol/kg	16
63 healthy subjects	unknown	8-oxodG	HPLC	172±79 pmol/kg	17
23 healthy subjects	unknown	8-oxodG	GC/MS	300±100 pmol/kg	18
53 healthy non-smokers vs.	40-64	8-oxodG	HPLC	213±84 pmol/kg	13
30 healthy smokers	40-64	8-oxodG	HPLC	320±99 pmol/kg	13
21 healthy non-smokers vs.	33_10	8-oxodG	HPLC	318±130 pmol/kg	15
12 healthy smokers	33_10	8-oxodG	HPLC	431±168 pmol/kg	15
300 g vegetable diet vs.	young	8-oxodG	HPLC	300±630 pmol/kg	19
300 g Brussels sprouts diet *	do.	do.	do.	210±490 pmol/kg	19
16 healthy non-smokers	35-50	8-oxodG	HPLC	666±202 pmol/kg	20
100% vs 60% energy in diet**	50	8-oxodG	GC/MS	345 vs. 110 pmol/kg	18
2 cancer patients before	57-59	8-oxodG	GC/MS	8-14 nmol	21
and after radiotherapy	do.	do.	do.	31-40 nmol	21

* n=5
** n=1

4 DISCUSSION

We tested an ELISA kit to determine 8-oxodG concentration in urine by comparing it to a multidimensional HPLC assay. The ELISA assay gave about eight fold higher results on average, however with high variation. The average level is compatible with the six fold higher levels found in another ELISA assay by Yin et al.[14] used on tissue, however. The higher degree of variation we observed in urine presumably relates to the larger variation in cross-reacting substances in urine due to differences in water intake, diet and environmental influence.

The HPLC assay is highly reproducible as can bee seen in Figure 2. In different studies both from our laboratory and those of others, urinary excretion rates in the same range have been found, Table 1, and we have been able to confirm increased excretion rates in different groups of smokers[13,15]. From a theoretical point of view HPLC is a highly selective and specific methodology. In the ELISA method specificity relies on the specificity of the reaction between the 8-oxodG and the antibody. DNA bases, RNA bases and oligonucleotides with or without oxidative modifications may cross-react. In larger series it could be of interest to determine differences between the ELISA detectable substances and the HPLC detectable substances to see if they represent other products of interest in relation to oxidative stress.

The commercially available assay is promising but presently it is too unspecific compared to the HPLC assay. Development of more specific antibodies may increase the specificity of the 8-oxodG ELISA assay, or combination with e.g. immunoaffinity-HPLC may make the ELISA assay applicable to molecular epidemiological studies.

Further advancement in the methodology - including verification that the 8-oxodG concentration in spot urine corrected by the urinary creatinine concentration is equivalent to the 8-oxodG level measured in 24 hours urine - is necessary for investigating the important question whether the oxidative DNA modification rate is predictive for development of degenerative and malignant diseases and ageing in man.

Acknowledgement

We than Genox for making the assay available. We thank BAT, UK and the Danish Environmental Programme for financial support. We also thank Bruce Ames for donating the 8-oxoGua.

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