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Original Research

Evaluation of DNA Damage in People Occupationally Exposed to Arsenic and Some Heavy Metals

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Abstract

Arsenic (As) is a human carcinogen with a high risk of cancer development in people exposed to it in industry and in the general environment. The mechanisms involved in arsenic carcinogenesis are still unknown. There is a hypothesis that reactive oxygen species (ROS) may play an important role in arsenic carcinogenesis. 8-hydroxydeoxyguanosine is a nucleotide form that results from oxidative DNA damage, which causes mutation *in vitro* and *in vivo*. So the occurrence of 8-OHdG (8-hydroxy-2'-deoxyguanosine) has been used to study damaging effects on DNA of ROS.

The aim of our study was to investigate whether oxidative damage of DNA, determined by elevated serum levels of 8-OHdG, could be observed in workers occupationally exposed to As and lead (Pb), and environmentally to cadmium (Cd).

According to the aim, a group of 47 copper smelters, working in the copper foundry "Legnica," and 20 matched non-exposed men were examined. Stressgen's StressXpress DNA Damage ELISA Kit was used for detection and quantitation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in serum samples.

The serum concentration of 8-OHdG was significantly higher in the study group than in the controls. No significant differences between serum 8-OHdG concentration in the group of exposed smokers, exposed non-smokers and smoking controls were found.

Moreover, no significant correlations between serum concentration of 8-OHdG and arsenic, lead and cadmium levels were observed, but people with the worst toxicological parameters showed higher 8-OHdG serum concentration.

The results suggest that:

1. chronic mixed exposure to arsenic, cadmium and lead may result in oxidative DNA damage in humans;
2. 8-OHdG serum level measurement may be a useful tool for biomonitoring in the case of mixed occupational exposure to these toxic metals and increased cancer risk.

Keywords: arsenic, compound occupational exposure, 8-OHdG, oxidative DNA damage

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Introduction

The mixed exposure for toxic substances (both clastogens and mutagens) occurs most often in the natural environment and in occupational settings. The example might be the coexistence of arsenic, lead and sulphur dioxide in the occupational environment of the copper smelters. There is often a synergistic interaction of these substances with smoke in smokers (as regards the incidence of lung cancer). The studies show that all these factors significantly increase the possibility of changes in DNA structure, which lead to development of neoplastic disease, also of occupational origin.

Many observations indicate that oxygen free radical events play an important role in enhancing carcinogenesis [1-5]. The exact mechanistic role that free radicals play is not understood at the present time. It is known that the premutagenic changes caused by oxidative DNA damage include a range of specifically oxidized purines and pyrimidines, alkali labile sites, strand breaks and instability formed directly or by repair processes [3]. Studies show that although all four bases are modified by reactive oxygen species (ROS), mutations are usually related to modification of GC base pair, while that of the AT base pair rarely leads to mutations. In human tumors, G to T transversion are the most frequent mutation in the p53 suppressor gene [3, 6]. However, a focal point where research effort is yelding an increasingly clearer view of the importance of the ROS in carcinogenesis are studies examining DNA base adduct formation. Research on oxygen free radical mediated 8-hydroxyguanine (8-OHG) and 8-hydroxy-2'deoxyguanosine (8-OHdG) formation in RNA and DNA have provided enhanced support for an important role of ROS in carcinogenesis.

There are several means of mediating 8-OHdG formation in RNA and DNA (Fig. 1).

One of the main reasons why 8-OHdG in DNA is considered as possibly an important factor in carcinogenesis is that its presence in DNA template causes alpha-polymerase to miscode incorporation of nucleotides in the replicated strand [1]. Supporting the *in vitro* results, recent data clearly indicate that 8-OHdG within DNA is mutagenic. The reason why the presence of 8-OHdG in DNA causes mutations is related to the perturbations it induces in the macromolecular structure [1, 4, 7]. Chemical modification of guanine, such as by oxidation, can affect the configuration of this base in DNA and alter its base-pairing properties. Such alterations can compromise the fidelity of a template containing this base during DNA replication. Introduction of an oxygen atom at the C8 position of G changes the electronic properties of this DNA base. 8-oxoG was first demonstrated to be mutagenic *in vitro* by Kuchino and coworkers [12]. The presence of 8-oxoG residues in DNA leads to a G:C to T:A transversion unless repaired prior to DNA replication [13]. Furthermore, many observations indicate a direct correlation between 8-oxo-guanine and 8-oxo-2'-deoxyguanosine formation and carcinogenesis.

In order for 8-OHdG to manifest its effects, it must remain in DNA for some period of time. Very little is known about the repair of 8-OHdG. Floyd et al. observed that thiourea removes 8-OHdG from isolated DNA, apparently without rupture of the DNA strands [1]. Kasai et al. found that 8-OHdG is excised from the oligonucleotides during the repair of DNA strand breaks created by ionizing irradiation [1, 8].

8-hydroxy-2'-deoxyguanosine (8-OHdG) is a modified nucleoside base, which is the most commonly studied and detected product of DNA damage that is excreted in urine upon DNA repair. Cigarette smoke, which is rich in carcinogens such as nitrosoamines and polycyclic aromatic hydrocarbons, causes accumulation of 8-OHdG. Urine obtained from smokers has a 4-10-fold elevation in altered nucleotides that are known to be produced by ROS. 8-OHdG and its analogs, 8-hydroxyguanosine and 8-hydroxyguanine (8-oxo-G), are linked to many degenerative diseases, not only to cancer. In the pathogenesis of atherosclerosis, diabetes, cystic fibrosis, atopic dermatitis, Parkinson's disease, Alzheimer's disease, Huntington's disease and depression, oxidative stress plays a significant role, so elevated levels of DNA damage products have been measured in a wide range of neurological conditions [3, 8-11]. In contrast, the consumption of vitamins C or E, lutein, vegetables, fruit, green tea and tomato sauce was correlated with a reduction in the amount of 8-OHdG in urine or leukocyte DNA [8].

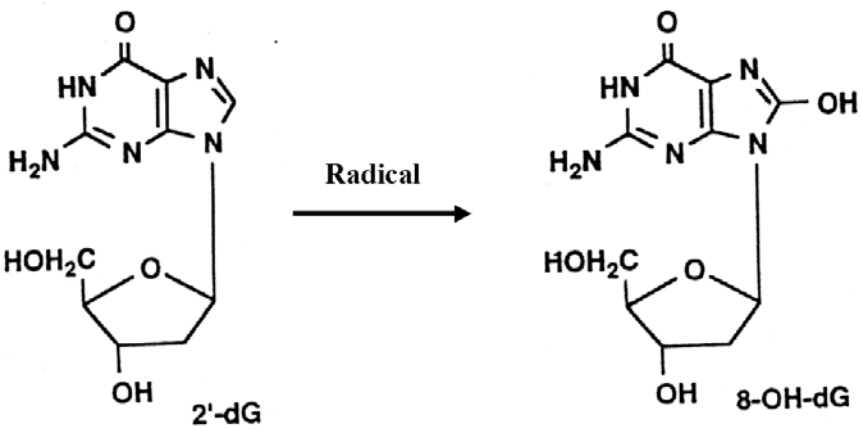

Fig. 1. 8-OHdG formation by oxygen radicals (from: H. Kasai: Environmental Mutagen Research, 10, 73-78, 1988).

Quantitatively, the 8-hydroxylated derivative of deoxyguanosine is one of most prevalent oxidized products of DNA generated *in vitro* under physiological conditions. This lesion also functions as a sensitive biomarker of oxidative DNA damage *in vivo*. Dose-dependent production of 8-OHdG also occurs in human cells in culture, whole animals, or bacteria exposed to ionizing radiation [12]. Furthermore, the excretion of 8-OHdG is correlated with age, metabolic rate, caloric intake, and antioxidant content of the diet.

The aim of this work was to investigate whether oxidative damage of DNA, determined by elevated serum levels of 8-OHdG, could be observed in workers occupationally exposed to arsenic (As) and lead (Pb), and environmentally to cadmium (Cd). The important question was the value of serum 8-OHdG concentration measurement in toxicological biomonitoring at the work place.

Material and Methods

Study Population

The study was conducted in a group of 47 healthy men, working in the "Legnica" copper foundry, employed in the furnace charge preparation and metallurgy departments, with an average age of 41, and 20 age and sex-matched non-exposed healthy men. (The control group was not large due to objective reasons.)

The study was performed according to an agreement between Wroclaw Medical University and the foundry. The evaluation of toxicological status and cancer risk assessment was an important part of medical care of copper foundry workers, so local ethics committee approval was not necessary in this case.

The method of individual measurement (dosimetry) was used in order to establish the noxious factors at the work place. The results of the measurements showed the presence of the arsenic and the minimal excess of the highest allowed lead concentration in the environment of the work place.

Laboratory Tests

Each worker underwent physical examination according to standard survey. In addition, the following lab tests were performed:

- blood Pb (lead) and Cd (cadmium) concentration;
- serum concentration of protecting metals, such as Cu (copper), and Zn (zinc);
- urine As (arsenic) concentration;
- blood FEP (free erythrocyte protoporphyrins) concentration;
- serum 8-OH-2'dG concentration.

The cigarette: year ratio (calculated as a product of cigarette day number and years of smoking) was evaluated in every smoking worker.

The blood samples for measurement of lead, FEP and cadmium were collected into a 2.7 cm^3 probe with EDTA, but Zn, Cu and 8-OH-2'dG concentrations were evaluated in 3 cm^3 of serum.

Methods

Blood lead concentration was measured by flameless atomic absorption spectrometry by means of measurement of absorbance at 283.3 nm wave with Zeeman background correction. The examination was done by SAA SOLAAR M6 of ThermoElemental company. The certified materials (BCR-194, - 195, -196 by IRMM, UE) were used as patterns.

Cadmium blood concentration was measured by flameless atomic absorption spectrometry by means of measurement of absorbance at 228.8 nm wave with Zeeman background correction. The examination was done by SAA SOLAAR M6 of ThermoElemental company. The certified materials (BCR-194, - 195, -196 by IRMM, UE) were used as patterns.

Serum copper and zinc concentration was measured by flame atomic absorption spectrometry by means of measurement of absorbance at 324.8 nm wave in acetylene and air flame with deuterium background correction. The examination was done by SAA SOLAAR M6 of ThermoElemental company. The pattern solutions, i.e. Single-Element Zinc (Copper) Standard 1,000 µg/ml certified by CPI International, were used.

Arsenic concentration was measured in acid (concentrated HNO_3) urine. The examination was done by SAA SOLAAR M6 with system PU 9360 Philips of hydride generation. A wave of 193.7 nm, deuterium background correction and electrical heated quartz atomization chamber were applied. The pattern solutions, i.e. Single-Element Arsenic Standard 1,000 µg/ml certified by CPI International, were used.

Concentration of free erythrocytes protoporphyrins (FEP) was evaluated by the Piomelli method [19].

Stressgen's StressXpress DNA Damage ELISA Kit was used for detection and quantitation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in serum samples.

Stressgen's StressXpress DNA Damage ELISA uses an 8-OHdG monoclonal antibody to bind, in a competitive manner, 8-OHdG in the sample, standard or pre-bound to the wells of the 96-well immunoassay plate. Anti-8-OHdG bound to 8-OHdG in the sample or standard are washed away while those captured by the immobilized 8-OHdG are detected with a secondary antibody: HRP conjugate. The assay is developed with tetramethylbenzidine substrate and the absorbance is measured in a microplate reader at 450 nm.

Statistical Analysis

Statistical analysis was performed with the STATISTICA 6.0 (StatSoft Polska, Kraków). Results were presented as mean value (x) and standard deviation (SD). Distribution of variables was checked using the Shapiro-Wilk test. Because of non-normal distribution of parameters, the differences between mean values were tested with nonpara-

Table 1. The mean values of study parameters in group of copper smelters and healthy controls.

N	Study group	age		Cu		Zn		Pb **		Cd *	
		x	sd	x	sd	x	sd	x	sd	x	sd
47	copper smelters	41.5	8.3	99.229	12.784	97.068	22.241	244.914	82.824	2.274	2.591
20	healthy control	40.8	9.0	101.055	14.318	99.555	9.835	36.275	12.683	0.825	0.773

As **		FEP		8-OhdG **		Cig-year	
x	sd	x	sd	x	sd	x	sd
56.668	39.513	45.665	28.957	180.023	14.562	311.521	241.493
3.124	1.690	33.260	8.511	164.050	15.915	249.333	180.274

* p<0.05
** p<0.001

Table 2. The mean values of study parameters in group of smokers and non-smokers.

N	Study group	smoking	age		Cu		Zn		Pb **	
			x	sd	x	sd	x	sd	x	sd
23	copper smelters	yes	40.1	8.5	99.382	12.187	96.330	21.432	258.565	92.34
24	copper smelters	no	42.9	8.0	99.083	13.592	97.775	23.428	231.833	72.09
9	healthy controls	yes	40.1	8.4	104.011	18.448	101.056	8.344	33.666	15.62
11	healthy controls	no	41.1	9.9	98.636	10.145	98.327	11.153	38.409	9.963

Cd ***		As **		FEP		8-OhdG****		Cig-year	
x	sd	x	sd	x	sd	x	sd	x	sd
3.017	2.187	65.404	51.830	47.208	27.370	179.434	13.506	311.521	241.493
1.561	2.787	48.295	20.088	44.187	30.916	180.587	15.777		
1.266	0.886	3.438	2.178	34.288	5.966	173.111	16.396	249.333	180.274
0.463	0.433	2.866	1.213	32.418	10.365	156.636	11.482		

** smoking copper smelters vs. smoking controls: p<0.001;
smoking copper smelters vs. non-smoking controls: p<0.001;
non-smoking copper smelters vs. smoking controls: p<0.001;
non-smoking copper smelters vs. non-smoking controls: p<0.001;
*** smoking copper smelters vs. smoking controls: p<0.05;
smoking copper smelters vs. non-smoking controls: p<0.01;
**** smoking copper smelters vs. non-smoking controls: p<0.001;
non-smoking copper smelters vs. non-smoking controls: p<0.001;
smoking controls vs. non-smoking controls: p<0.05.

metric tests for unpaired variables – the Mann-Whitney U test or the ANOVA Kruskal-Wallis test. Statistical differences were assessed using the post hoc Newman-Keuls test. The analysis of correlation was performed to determine the association between the tested variables. Values of p<0.05 were considered statistically significant.

Results

The serum concentration of 8-OHdG was significantly higher in the study group than in the controls (180.02 ng/ml ±14.56 v. 164.05 ng/ml ±15.91; p< 0.001) (Table 1).

A significantly higher serum concentration of 8-OHdG was observed in the groups of smoking and nonsmoking exposed men vs. nonsmoking controls (179.43 ng/ml ±13.50; 180.58 ng/ml ±15.77; v. 156.63ng/ml ± 11.48 p< 0.001; p< 0.001; p< 0.05, respectively). There were no significant differences between serum 8-OHdG concentration in group of exposed smokers, exposed nonsmokers and smoking controls. Therefore, arsenic, lead, cadmium and nicotine might damage DNA independently from a similar strength (Table 2).

The statistically significant difference between serum 8-OHdG concentration in the group of workers with highest lead level and healthy controls indicates that lead (or its tissue deposits) may be an important oxidative factor (Table 3).

Table 3. The mean values of study parameters regarding lead blood concentration.

N	Pb median (228ug/ml)	age		Cu		Zn **		Pb *		Cd ***	
		x	sd	x	sd	x	sd	x	sd	x	sd
24	> median	40.7	9.1	103.412	12.612	106.058	24.781	309.292	61.738	2.798	3.211
23	< median	42.4	7.3	94.865	11.681	87.687	14.565	177.739	33.465	1.727	1.628
20	healthy controls	40.8	9.0	101.055	14.318	99.555	9.836	36.275	12.684	0.825	0.773

As *		FEP		8-OhdG*		Cig-year	
x	sd	x	sd	x	sd	x	sd
52.008	32.610	48.367	25.128	181.504	15.578	251.250	237.536
61.530	45.874	42.847	32.815	178.478	13.594	377.273	239.002
3.124	1.691	33.260	8.512	164.050	15.916	249.333	180.275

* > median vs. controls: p<0.001; < median vs. controls: p<0.001;
** > median vs. controls: p<0.05; > median vs. < median: p<0.001;
*** > median vs. controls: p<0.01.

Table 4. The mean values of study parameters regarding arsenic urine concentration.

N	As median (44.9ug/g creat)	age		Cu		Zn		Pb **	
		x	sd	x	sd	x	sd	x	sd
24	> median	40.9	8.8	97.300	13.925	95.420	21.389	224.708	78.348
23	< median	42.1	7.7	101.243	11.431	98.786	23.451	266.000	83.760
20	healthy controls	40.8	9.1	101.055	14.318	99.555	9.835	36.275	12.683

Cd ***		As **		FEP		8-OhdG*		Cig-year	
x	sd	x	sd	x	sd	x	sd	x	sd
1.122	0.836	79.454	44.232	44.766	32.986	181.416	16.026	240.416	215.495
3.476	3.217	32.891	7.850	46.604	24.776	178.569	13.058	389.090	254.035
0.825	0.773	3.124	1.690	33.260	8.511	164.050	15.915	249.333	180.274

* > median vs. controls: p<0.001; < median vs. controls: p<0.001;
** > median vs. controls: p<0.001; < median vs. controls: p<0.001; > median vs. < median: p<0.05;
*** < median vs. controls: p<0.001; > median vs. < median: p<0.001.

8-OHdG serum concentration was significantly higher in the group of copper smelters with highest arsenic urine levels in comparison with control group, but there was no significant difference between 8-OHdG level in the subgroups of various arsenic concentrations in urine (Table 4).

The analysis of serum 8-OHdG concentration in subgroups of different cadmium blood levels revealed that concentration of 8-OHdG was highest in people with significantly increased cadmium blood levels, but (analogically to the arsenic) there was no significant difference between its concentration in the group of workers with higher and lower cadmium load in an organism (Table 5).

Moreover, it was observed that in all analyzed subgroups of study population people with the worst toxicological parameters were significantly younger and absorbed more than one toxic metal simultaneously. It might suggest that young and potentially healthy people present the highest inclination to absorption and accumulation of toxic metals. It might be evidence of gradual increasing constitutional tolerance of people over 40 to toxic and carcinogenic influence of some metals. Although it might be possible that younger workers have left the workplace because of increased incidence of illnesses due to higher metals absorption and this group is not representative enough.

There were no significant correlations between serum concentration of 8-OHdG and arsenic urine concentrations, or cadmium and lead blood concentration in the study group (Table 6).

Discussion

During the last two decades, chemical and cellular studies have provided a variety of contributions to extend our understanding for metal-induced carcinogenesis. Although mutations on genome DNA which are capable of activating oncogenes or inactivating tumor suppressors, are tradition-

Table 5. The mean values of study parameters regarding the cadmium blood concentration.

N	Cd median (1.20ug/ml)	age		Cu		Zn		Pb **	
		x	sd	x	sd	x	sd	x	sd
24	> median	39.5	8.4	99.63	11.349	96.233	22.441	255.58	84.21
23	< median	43.7	7.5	98.82	14.378	97.939	22.501	233.78	81.71
20	healthy controls	40.8	9.1	101.06	14.318	99.555	9.836	36.28	12.68

Cd ***		As **		FEP		8-OhdG**		Cig-year	
x	sd	x	sd	x	sd	x	sd	x	sd
3.935	2.735	56.992	51.435	46.612	28.311	178.84	13.120	331.84	253.77
0.540	0.252	56.330	22.328	44.678	30.221	181.26	16.134	215.00	161.97
0.825	0.773	3.124	1.691	33.260	8.512	164.05	15.916	249.33	180.27

** > median vs. controls: $p<0.001$; < median vs. controls: $p<0.001$;
*** > median vs. controls: $p<0.001$; > median vs. < median: $p<0.001$.

Table 6. Correlations in the study group.

	age	Mg	Ca	Cu	Zn	Pb	Cd	Cd u	As	FEP	8-OHdG	cig-year
age	1.00	-0.01	0.21	0.03	**-0.62**	-0.14	-0.15	**0.42**	-0.24	-0.38	-0.26	**0.51**
Mg	-0.01	1.00	0.33	0.29	0.15	-0.35	0.26	0.24	0.08	0.06	-0.09	0.27
Ca	0.21	0.33	1.00	0.27	-0.07	-0.01	0.00	**0.44**	-0.20	-0.16	0.15	0.08
Cu	0.03	0.29	0.27	1.00	0.05	-0.04	0.12	0.28	-0.22	-0.10	**0.65**	**0.48**
Zn	**-0.62**	0.15	-0.07	0.05	1.00	0.19	0.09	-0.29	0.18	0.27	0.25	-0.32
Pb	-0.14	-0.35	-0.01	-0.04	0.19	1.00	-0.11	-0.35	-0.15	0.32	0.30	-0.31
Cd	-0.15	0.26	0.00	0.12	0.09	-0.11	1.00	-0.01	-0.36	-0.19	-0.32	0.30
Cd u	**0.42**	0.24	**0.44**	0.28	-0.29	-0.35	-0.01	1.00	0.02	-0.34	0.13	0.14
As	-0.24	0.08	-0.20	-0.22	0.18	-0.15	-0.36	0.02	1.00	**0.49**	0.05	-0.35
FEP	-0.38	0.06	-0.16	-0.10	0.27	0.32	-0.19	-0.34	**0.49**	1.00	-0.04	-0.38
8-OHdG	-0.26	-0.09	0.15	**0.65**	0.25	0.30	-0.32	0.13	0.05	-0.04	1.00	0.02
cig-year	**0.51**	0.27	0.08	**0.48**	-0.32	-0.31	0.30	0.14	-0.35	-0.38	0.02	1.00

Marked correlations are significant at $p < 0.05000$.

ally considered to be the crucial factor for cancer initiation, other events, such as transcription activation, recombination and oncogene amplification, which may or may not require DNA damage, also contribute to the tumor initiation process [2].

Accumulating evidence suggests that ROS plays an important role in mediating metal-induced cellular responses and carcinogenesis.

8-OHdG is a byproduct of ROS damage to DNA, which can cause mutation of G:C to A:T if it remains in the DNA at the time of replication [3, 7]. 8-OHdG in urine is a widely accepted marker of oxidative DNA damage and oxidative stress. Normal DNA repair removes 8-OHdG adducts, which are excreted and measurable in urine, blood and tissues.

Many types of life-style factors that either generate or scavenge oxygen radicals may affect the level of oxidative DNA damage of each individual. Moderate physical exercise, high BMI, and age showed significant reducing effects on the 8-OH-dG level, while low meat intake, smoking, and day-night shift work increased its level [14].

Background levels of 8-OHdG and its derivatives have been analyzed in different human tissues [15]. Kasai mentioned that more than 40 papers about human samples have been published so far [15, 16]. In liver samples from humans with chronic hepatitis, higher levels of 8-OHdG were detected, suggesting that oxygen radicals are involved in human hepatocarcinogenesis. Increased 8-OHdG levels have been detected in the DNA obtained from a central site in the lungs of smokers or in the nuclei of human oral

mucosa cells from smokers. A positive correlation between 8-OHdG concentration and the Brinkman index (number of cigarettes smoked per day x number of years of the habit) was obtained for smokers and ex-smokers, as well as for the 8-OHdG and the number of cigarettes smoked per day. A higher level of 8-OHdG was also detected in the peripheral part of the lungs from lung cancer patients as compared to non-cancer controls [16]. It is worth mentioning that many G:C to A:T transversions, induced by 8-OHdG, have been detected in p53 genes isolated from human lung and liver cancers [3, 6].

Results of many studies conducted thus far indicate that 8-OHdG is a sensitive biomarker to asses the extent of DNA damage and repair in both clinical and occupational settings. In workers exposed occupationally to benzene, a dose-response relationship was found between personal exposure to benzene and urinary 8-OHdG concentration [17]. Workers exposed to asbestos, chromium, rubber, azo-dye industries and diesel particulate exhaust also were found to have significantly higher urinary 8-OHdG levels than nonexposed people [5, 16, 18, 20-22]. Finally, in the study of Jee and coworkers, 8-OHdG was defined as a biomarker of oxidative DNA injury after exposure to high levels of metal-containing particulate matter, concentration of which significantly increased in urine in a relatively young and healthy cohort of boilermakers [18]. However, direct measurements of the 8-OHdG level are difficult, because of the limitations of current methodologies and variation between different cell types. The range of the analyzed 8-OHdG levels differs 10-1000-fold depending on the method used. Higher levels of 8-OHdG were detected in females as compared to males and in fat people as compared to lean people [16]. Some authors reported that the 8-OHdG concentration in human urine increases after the inhalation of car exhaust.

In 1986 Floyd developed a sensitive method to analyze 8-OHdG using an electrochemical detector with high performance liquid chromatography (HPLC-ECD). This method revealed that various ROS-forming carcinogens induce an increase of 8-OHdG in cellular DNA [10]. Since then, HPLC-ECD has become the most popular (and probably best) method to evaluate 8-OHdG concentrations in human cells [6, 23]. An alternative approach to asses oxidative DNA damage is the measurement of urinary excretion of 8-oxoG and 8-oxo-2'dG. It is generally accepted that the products of repair of 8-oxoG in cellular DNA are excreted into the urine without further metabolism, and that the amount of the modified base or nucleoside excreted into urine should represent the average rate of DNA damage in the whole body. Kasai found [8] that the urinary 8-hydroxyguanine (8-OH-Gua, 8-oxoGua) free base, analyzed by the HPLC method, is a better marker than 8-OHdG for oxidative stress, because of its sensitivity and specificity. Kim et al. [18] used this marker to evaluate oxidative stress effects in human lung alveolar epithelial cells induced by crocidolite asbestos.

Another form of DNA damage is 5-OH-2'cytosine, causing C:G to A:T mutations with high frequency and detected by HPLC. It was also found that 2-OHdATP and glyoxal, formed in DNA by oxygen radicals, induces G:C and A:T mutations in bacterial and mammalian cells [16].

There are a lot of papers suggesting that oxidative stress is an important mechanism of arsenic toxic action in human cells [2, 5, 24-27]. Breton and coworkers [7] mentioned that in a cross-sectional study of Chinese community residents exposed to drinking water arsenic, oxidative stress level, measured by mean serum levels of lipid peroxide, were significantly higher among the arsenic-exposed group. Non-protein sulfhydryl groups were also inversely correlated with mean serum arsenic levels. Several epidemiologic studies have also demonstrated an association between arsenic and 8-OHdG [25, 26]. In addition, skin tissue studies have shown significant differences in 8-OHdG concentration when comparing arsenic exposed and unexposed skin samples [7].

Most investigators use urinary level of 8-OHdG as the marker of oxidative stress induced by some carcinogens. The results of our studies are evidence that serum concentration of 8-OHdG may also be a useful and not expensive tool for evaluation of DNA damage processes in people exposed to toxic and carcinogenic metals, especially in a case of mixed occupational exposure.

A relationship between a smoking habit and the development of some cancers is obvious. The risk is higher if smoking people are exposed to several carcinogenic compounds simultaneously. A lot of observations make it likely that the excess cancer risk in the smelter environment is multifactorial in character, involving interactions between both carcinogenic and anticarcinogenic factors [28]. For example a dose-response relationship for lung cancer was observed with exposure to arsenic and SO_2. When cigarette smoking data were included with arsenic and SO_2 exposure data in a nested case-control analysis, only smoking and arsenic were statistically significant factors [29]. According to De Palma et al., tobacco smoke is a main factor affecting the concentration levels of cadmium, lead and, to a lesser extent, of nickel in the lung tissues of non-small cell lung cancer patients [30].

In studies of Jones, Atkin et al. [31] no significant associations could be found between lung cancer mortality and simple cumulative exposure to arsenic, lead, cadmium, antimony and polonium-210. When cumulative exposures were weighted according to time since exposure and attained age, significant associations were found between lung cancer mortality and exposures to arsenic, lead and antimony. In conclusion the authors confirm that the excess of lung cancer mortality in the cohort of arsenic-, lead- and cadmium-exposed tin smelters can most plausibly be explained if arsenic is the principal occupational carcinogen (for which the excess relative risk diminishes with time since exposure and attained age), and if there is a contribution to excess mortality from an enhanced prevalence of smoking within the cohort [31].

The results of Chia and coworkers study [32] on secondary copper and zinc recovery plant smelters showed that the older subjects exhibited significantly lower levels

of 8-OH-dG than younger subjects. Their investigation also showed that working departments were related to DNA strand breakage levels of nonsmokers, but the observation become negligible in smokers. So it was implicated that cigarette type might affect 8-OH-dG levels in young people exposed to toxic metals.

In our study the serum 8-OHdG concentration was higher in the study group than in the healthy controls. It should be underlined that in the study population arsenic, lead and cadmium levels were all high, so all compounds influenced 8-OHdG concentration and it is not possible to determine which one was most toxic. In our opinion, populations like this should be carefully observed, because the risk of metals toxicity and carcinogenicity significantly increases in cases of mixed exposure. We suggest that 8-OHdG serum concentration may be a useful marker for monitoring genetic results of metal exposure.

Moreover, there was no significant difference between serum 8-OHdG concentrations in a group of exposed smokers, exposed nonsmokers and smoking controls. The marked difference was observed only in smoking or non-smoking exposed men and non-smoking controls. It might suggest that arsenic, lead, cadmium and nicotine are strong oxidative factors damaging DNA independently with the similar strength and the smoking habit does not augment the DNA oxidative damage in the case of simultaneous exposure to toxic metals.

The lack of significant correlation between serum 8-OHdG level and concentrations of toxic metals is an unexpected observation. In our opinion, this fact should be verified at the next stage of study, using HPLC as an alternative method for 8-OHdG measurement.

There are two universal pathways of repairing DNA impairments caused by reactive oxygen species: base excision repair (BER) and nucleotide excision repair (NER). NER is more complicated than the BER process and requires ATP as an energy source. Data resulting from in vitro studies indicate that NER is a comprehensive repair mechanism of DNA damage generated by different carcinogens [33]. In our present study we assessed NER processes indirectly by their product – 8OHdG – serum concentration. The direct evaluation of NER or BER, involving specific nucleases or glycosylases activity measurement, would be an alternative and valuable method. Results of Pu and coworkers study [34] suggest that 8-oxoguanine DNA glycosylase (OGG) and MutY glycosylase (MYH) are involved in the incision of arsenite-induced DNA adducts. Both enzymes are bifunctional glycosylases with associated lyase activity and excise oxidized bases, and then nick the DNA strand 3' to the apurinic/apyrimidinic site by beta-elimination mechanism. Cells or individuals with low activity of these enzymes may be more susceptible to arsenic toxicity and carcinogeity.

Conclusions

1. Chronic mixed exposure to arsenic, lead and cadmium may result in oxidative DNA damage in humans.
2. 8-OHdG serum level measurement may be a useful marker for biomonitoring in the case of mixed occupational exposure to toxic metals and increased cancer risk.
3. In healthy smoking people, 8-OHdG concentration is significantly higher than in non-smokers, which is evidence of DNA oxidative damage due to nicotine.
4. The lack of differences between 8-OHdG serum levels in exposed smokers and exposed non-smokers might be evidence that toxic and mutagenic metals cause oxidative stress in human DNA as much as nicotine.

References

1. FLOYD R.A. The role of 8-hydroxyguanine in carcinogenesis – commentary. Carcinogenesis, **11**, (9), 1447, **1990**.
2. WANG S., XIANGLIN S. Molecular mechanisms of metal toxicity and carcinogenesis. Molecular and Cellular Biochemistry, **222**, 3, **2001**.
3. WARIS G., AHSAN H. Reactive oxygen species: role in the development of cancer and various chronic conditions. Journal of Carcinogenesis, **5**, 14, **2006**.
4. LOFT S., POULSEN H.E. Cancer risk and oxidative DNA damage in man. J Mol. Med., **74**, (6), 297, **1996**.
5. HAMILTON J.W., KALTREIDER R.C., BAJENOVA O.V., IHNAT M.A., MCCAFFREY J., TURPIE B.W. et al: Molecular basis for effets of carcinogenic heavy metals on inducible gene expression. Environ. Health Perspect., **106,** (4), 1005, **1998**.
6. AKCAY T., SAYGILI I., ANDICAN G., YALCIN V. Increased formation of 8-OHdG in peripheral blood leukocytes in bladder cancer. Urol. Int., **71**, 271, **2003**.
7. BRETON C.V., KILE M.L., CATALANO P.J., HOFFMAN E., QUAMRUZZAMAN Q., RAHMAN M. GSTM I and APE I genotypes affect arsenic-induced oxidative stress: a repeated measures study. Environ. Health, **6**, 39, **2007**.
8. KASAI H., KAWAI K. Oxidative DNA damage: mechanisms and significance in health and disease. Antioxidants and Redox Signaling, **8**, (5, 6), 981, **2006**.
9. StressXpress by Stressgen Bioreagents Corp.: DNA damage ELISA Kit for the detection and quantitation of 8-OHdG in urine and serum samples. Catalog Number EKS-350, pp. 2-4.
10. FORLENZA M.J., MILLER G.E. Increased serum levels of 8-OHdG in clinical depression. Psychosomatic Medicine, **68**, 1, **2006**.
11. WU L.L., CHIOU C.C., CHANG P.Y., WU J.T. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin. Chim. Acta, **339**, (1-2), 1, **2004**.
12. GUYTON K.Z., KENSLER T.W. Oxidative mechanisms in carcinogenesis. British Medical Bulletin, **49**, 523, **1993**.
13. FOKSIŃSKI M., GACKOWSKI D., ROZALSKI R., OLIŃSKI R. Cellular level of 8-oxo-2'-deoxyguanosine in DNA does not correlate with urinary excretion of the modified base nucleoside. Acta Biochimica Polonica, **50**, (2), 549, **2003**.
14. KASAI H., IWAMOTO-TANAKA N., MIYAMOTO T., KAWANAMI K., KAWANAMI S., KIDO R., IKEDA M. Life style and urinary 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage: effects of exercise, working conditions, meat intake, body mass index and smoking. Jpn J Cancer Res., **92**, (1), 9, **2001**.

15. KASAI H. Analysis of a form of oxidative DNA damage, 8-OHdG, as a marker of cellular oxidative stress during carcinogenesis. Mutation Research, **387**, (3), 147, **1997**.
16. LAGORIO S., TAGESSON C., FORASTIERE F., IAVARONE I., AXELSON O., CARERE A. Exposure to benzene and urinary concentrations of 8-OHdG, a biological marker of oxidative damage to DNA. Occup. Environ. Med., **51**, (11), 739, **1994**.
17. JEE Y. KIM, SUTAPA M., LONG N., CHRISTIANI D.C. Urinary 8-hydroxy-2'-deoxyguanosine as a biomarker of oxidative DNA damage in workers exposed to fine particulates. Environ. Health Perspect., **112**, (6), 666, **2004**.
18. KIM H. N., MORIMOTO Y., TSUDA T., OOTSUYAMA Y., HIROHASHI M., HIRANO T. Changes in DNA 8-hydroxyguanine levels, 8-hydroxyguanine repair activity, and hOGG1 and hMTH1 mRNA expression in human lung alveolar epithelial cells induced by crocidolite asbestos. Carcinogenesis, **22**, (2), 265, **2001**.
19. PIOMELLI S. A micromethod for free erythrocyte porphyrins: the FEP test. J Lab Clin Med; **81**, 932, **1973**.
20. HARRI M., SVOBODA P., MORI T., MUTANEN P., KASAI H., SAVELA K. Analysis of 8-OhdG among workers exposed to diesel particulate exhaust: comparison with urinary metabolites and PAH air monitoring. Free Radic. Res., **39**, (9), 963, **2005**.
21. KUO H.W., CHANG S.F., WU K.Y., WU F.Y. Chromium (VI) induced oxidative damage to DNA: increase of urinary 8-OHdG concentrations among electroplating workers. Occup. Environ. Med., **60**, 590, **2003**.
22. TAKEUCHI T., NAKAJIMA M., OHTA Y., MURE K., TAKESHITA T., MORIMOTO K. Evaluation of 8-OHdG, a typical oxidative DNA damage, in human leukocytes. Carcinogenesis, **15**, (8), 1519, **1994**.
23. PI J., YAMAUCHI H., KUMEGAI Y., SUN G., YOSHIDA T., AIKAWA H., HOPENHAYN-RICH C. Evidence for induction of oxidative stress caused by chronic exposure of chinese residents to arsenic contained in drinking water. Environ. Health Perspect., **110**, (4), 331, **2002**.
24. LANTZ R.C., HAYS A.M. Role of oxidative stress in arsenic-induced toxicity. Drug Metab. Rev., **38**, (4), 791, **2006**.
25. YAMAUCHI H., AMINAKA Y., YOSHIDA K., SUN G., PI J., WAALKES M.P. Evaluation of DNA damage in patients with arsenic poisoning: urinary 8-hydroxydeoxyguanine. Toxicol. Appl. Pharmacol., **198**, (3), 291, **2004**.
26. BURGESS J.L., MEZA M.M., JOSYULA A.B., POPLIN G.S., KOPPLIN M.J., MCCLELLEN H.E. Environmental arsenic exposure and urinary 8-OHdG in Arizona and Sonora. Clin. Toxicol., **45**, (5), 490, **2007**.
27. ANDREW A.S., BURGESS J.L., MEZA M.M., DEMIDENKO E., WAUGH M.G., HAMILTON J.W. et al: Arsenic exposure is associated with decreased DNA repair in vitro and in individuals exposed to drinking water arsenic. Environ. Health Perspect., **114**, (8), 1193, **2006**.
28. GERHARDSSON L., NORDBERG G.F. Lung cancer in smelter workers – interactions of metals as indicated by tissue levels. Scand J Work Environ Health., **19,** (1), 90, **1993**.
29. ENTERLINE P.E., MARSH G.M., ESMEN N.A., HENDERSON V.L., CALLAHAN C.M., PAIK M. Some effects of cigarette smoking, arsenic, and SO_2 on mortality among US copper smelter workers. J Occup Med. **29**, (10), 831, **1987**.
30. DE PALMA G., GOLDONI M., CATALANI S., CARBOGNANI P., POLI D., MOZZONI P., ACAMPA O., INTERNULLO E., RUSCA M., APOSTOLI P. Metallic elements in pulmonary biopsies from lung cancer and control subjects. Acta Biomed., **79**, (1), 43, **2008**.
31. JONES S.R., ATKIN P., HOLROYD C., LUTMAN E., BATLLE J.V., WAKEFORD R., WALKER P. Lung cancer mortality at a UK tin smelter. Occup Med, **57**, (4), 238, **2007**.
32. CHIA T., HSU CH.Y., CHEN H.L. Oxidative damage of workers in secondary metal recovery plants affected by smoking status and joining the smelting work. Industrial Health **46**, 174, **2008**.
33. ROSZKOWSKI K. Repairing mechanisms of oxidative DNA damage. Współczesna Onkologia, **6**, 6, 360, **2002**.
34. PU Y.S., JAN K.Y., WANG T.C., WANG A.S., GURR J.R. 8-oxoguanine DNA glycosylase and MutY homolog are involved in the incision of arsenite-induced DNA adducts. Toxicological Sciences, **95**, 2, 367, **2007**.