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Markers of nucleic acids and proteins oxidation among office workers exposed to air pollutants including (nano)TiO_2 particles

Daniela Pelclova [1], Vladimir Zdimal [2], Petr Kacer [3], Stepanka Vlckova [1], Zdenka Fenclova [1], Tomas Navratil [4], Martin Komarc [5,6], Jaroslav Schwarz [2], Nadezda Zikova [2], Otakar Makes [2], Sergey Zakharov [1]

1 Charles University and General University Hospital in Prague, 1st Faculty of Medicine, Department of Occupational Medicine, Prague, Czech Republic
2 Institute of Chemical Process Fundamentals of the CAS CR, v.v.i., Prague, Czech Republic
3 Institute of Chemical Technology Prague, Czech Republic
4 J. Heyrovský Institute of Physical Chemistry of the CAS CR, v.v.i., Prague, Czech Republic
5 Charles University, Faculty of Physical Education and Sport, Department of Kinanthropology and Humanities, Prague, Czech Republic
6 Charles University, 1st Faculty of Medicine, Institute of Biophysics and Informatics, Prague, Czech Republic

Correspondence to: Prof. Daniela Pelclová, MD., PhD.
Department of Occupational Medicine
Charles University and General University Hospital
Na Bojisti 1, 120 00 Prague, Czech Republic.
TEL: +420 224 964 532; E-MAIL: daniela.pelclova@lf1.cuni.cz

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Abstract

OBJECTIVES: Experimental studies using nanoscale TiO_2 have documented lung injury, inflammation, oxidative stress, and genotoxicity. Human health data are extremely scarce.

METHODS: In exhaled breath condensate (EBC) and urine of 22 office employees occupationally exposed to TiO_2 during their visit in the production workshops for average 14±9 min/day a panel of biomarkers of nucleic acids and proteins oxidation was studied, specifically 8-hydroxy-2-deoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-OHG), 5-hydroxymethyl uracil (5-OHMeU), o-tyrosine (o-Tyr), 3-chlorotyrosine (3-ClTyr), and 3-nitrotyrosine (3-NOTyr). Examination was performed also in 14 comparable controls.

RESULTS: The median respirable TiO_2 mass concentration in the workshops was 0.40 mg/m^3, median number concentration was 2.32×10^4 particles/cm^3 with 80% of the particles being <100 nm in diameter. All 6 markers of oxidation were elevated in EBC in factory office employees relative to controls ($p<0.01$). Significant association was found between their job in TiO_2 production plant and 5 markers of oxidation (except 3-NOTyr) in the EBC in multivariate analysis. No elevation of markers was detected in the urine.

CONCLUSION: This pilot study suggests that even short nanoTiO_2 exposure may lead to pulmonary oxidative stress; however this effect may be short-term and reversible. The clinical significance of these findings is unclear and more studies are needed.

ORIGINAL ARTICLE

Abbreviations:

3-ClTyr	- 3-chlorotyrosine
3-NOTyr	- 3-nitrotyrosine
5-OHMeU	- 5-hydroxymethyl uracil
8-isoprostane	- 8-isoProstaglandine F2α
8-OHdG	- 8-hydroxy-2′-deoxyguanosine
8-oxodG	- 8-oxo-7,8-dihydro-2′-deoxyguanosine
8-OHG	- 8-hydroxyguanosine
BMI	- body mass index
EBC	- exhaled breath condensate
LC-ESI-MS/MS	- liquid chromatography - electrospray ionization - tandem spectrometry
o-Tyr	- o-tyrosine

INTRODUCTION

Experimental data show that nanoparticles influence lung physiology; they have adverse effects due to a larger surface area and higher predicted pulmonary deposition and animals exposed to a high dose of nanoTiO_2 developed pulmonary emphysema, epithelial cell apoptosis related to oxidative stress (Chang *et al.* 2013; Li *et al.* 2013). 8-hydroxy-2'-deoxyguanosine (8-OHdG or 8-oxodG), 8-hydroxyguanosine (8-OHG) which originate from guanine, and 5-hydroxymethyl uracil (5-OHMeU) formed from thymine reflect oxidation of nucleic acids (Li *et al.* 2013). In proteins, o-tyrosine (o-Tyr) may be generated from phenylalanine, and 3-chlorotyrosine (3-ClTyr) and 3-nitrotyrosine (3-NOTyr) from tyrosine (Syslova *et al.* 2014).

In workers exposed to nanoparticles during production of white TiO_2 and red/brown Fe oxide pigments, markers of oxidation of nucleic acids, proteins, and lipids were highly elevated in their exhaled breath condensate (EBC) (Pelclova *et al.* 2016a, Pelclova *et al.* 2016b). In this pilot study, office workers exposed to TiO_2 for a short period of the shift during the control of production were studied using identical methods.

MATERIAL AND METHODS

Twenty-two male office employees were examined. They visited for a daily average of 0.23±0.15 hours (14±9 min) the production workshops where TiO_2 pigment was manufactured. In addition, 14 control subjects not employed in the factory were examined; they worked in the offices as healthcare personnel and technical staff. The study was carried out according to the Helsinki Declaration. The Ethical Committee of the Charles University approved the study. All participants signed the informed consent.

The EBC samples were collected using the Ecoscreen Turbo DECCS, Jaeger, Germany. All subjects breathed tidally for 15 minutes through a mouthpiece connected to the condenser (–20°C) while wearing a nose-clip (Horvath *et al.* 2005). Urine spot samples were given. Oxidation products of nucleic acids and proteins were analyzed by liquid chromatography-electrospray ionization-tandem spectrometry (LC-ESI-MS/MS) (Syslova *et al.* 2010).

Aerosol measurements were carried out for mapping and localisation of the main sources of TiO_2 aerosol particles using a portable particle number concentration monitor P-TRAK, and a portable monitor of particle mass concentrations, DustTRAK DRX (both TSI Inc, USA). The dynamics of aerosol particle number size distributions (PSD) were monitored by a scanning mobility particle sizer (SMPS), model 3936 L, TSI Inc, USA, as well as an aerodynamic particle sizer (APS), model 3321, TSI Inc, USA. The details of the workplace aerosol measurements were described in our publication on the worker´s results (Pelclova *et al.* 2016a). Statistical analysis of the two groups under study was done using chi-square test, t-test or Mann-Whitney test, where the specific test was chosen based on the type and distributional properties of a given variable. Methods of correlation and regression analysis were used to assess the relationship between the variables of interest. All analyses were conducted using SPSS V.22.0 (SPSS, Inc., Chicago, Illinois, USA).

RESULTS

The groups of subjects were comparable in most characteristics**,** as shown in Table 1. The levels of markers of oxidation were higher in the office employees from TiO_2 producing plant, as can be seen in Figure 1. TiO_2

Tab. 1. Characteristics of the groups of subjects.

	(TiO_2) Office Employees	Controls
N	22	14
Age (years)	44.3±3.9	38.5±4.5
Exposure (years)	15.5±3.6	--
Smoking (yes/no)	1 (4.5%)	7 (50.0%)**
Alcohol daily (yes/no)	22 (100.0%)	14 (100.0%)
Chronic bronchitis (yes/no)	0 (0%)	3 (21.4%)*
Asthma (yes/no)	2 (9.1%)	1 (7.1%)

*p<0.05; **p<0.01

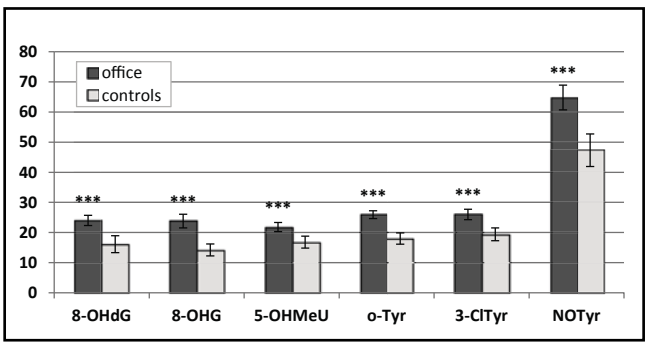

Fig. 1. Markers of oxidation of nucleic acids and proteins in EBC (pg/ml). Mean and 95% confidence interval (C.I.) are shown.

Tab. 2. Multiple regression analysis (unstandardized regression coefficients with 95% C.I. in brackets) of employment in the office of TiO_2 producing plant, age, smoking, body mass index, and markers in EBC

	8-OHdG	8-OHG	5-OHMeU	o-Tyr	3-ClTyr	3-NOTyr
TiO_2 – Office (Yes/No)	8.47** (3.12, 13.82)	11.05*** (5.46, 16.64)	4.38* (0.44, 8.31)	6.03** (2.33, 9.73)	8.20** (3.56, 12.84)	10.52 (–0.95, 21.99)
Age (years)	–0.04 (–0.23, 0.15)	–0.07 (–0.27, 0.13)	0.05 (–0.09, 0.19)	–0.04 (–0.18, 0.09)	0.05 (–0.11, 0.22)	0.29 (–0.12, 0.70)
Smoking (Yes/No)	–2.11 (–7.22, 3.00)	–1.20 (–6.54, 4.14)	0.41 (–3.35, 4.17)	–0.54 (–4.08, 2.99)	2.72 (–1.71, 7.16)	–2.03 (–13.00, 8.93)
BMI (kg/m^2)	–0.28 (–0.71, 0.16)	0.20 (–0.25, 0.66)	0.17 (–0.15, 0.49)	0.07 (–0.23, 0.37)	0.03 (–0.34, 0.41)	–0.24 (–1.17, 0.69)

*p<0.05; **p<0.01; and ***p<0.001

aerosol measurements in the workplace area found that median total mass TiO_2 concentration was 0.40 mg/m^3 and the median number of concentrations 2.32×10^4 particles/cm^3; about 80% of particles were smaller than 100 nm in diameter (Pelclova *et al.* 2016c).

There was no correlation of the markers with systemic disorders (hypertension, increased cholesterol, cancer, diabetes) and respiratory diseases, except for asthma and 3-NOTyr (p<0.05). Multiple regression analysis confirmed an association solely between occupational exposure in the plant producing (nano)TiO_2 and five markers of oxidation of nucleic acids and proteins, as shown in Table 2. The association was strongest for 8-OHG.

DISCUSSION

This study follows the study in the workers producing white TiO_2 pigment, which found elevations of markers of oxidative stress in the EBC of the workers who have spent about 40% of their shift (2.5–3.7 hours) in the workshops (the rest of the time in the operating room) (Pelclova *et al.* 2016a). Obviously, the markers in the office employees were significantly lower (p<0.01) than in the production workers and their lung functions were in the reference range (Pelclova *et al.* 2016b).

Until now, the human studies both in the production workers and office employees are very limited (Liou *et al.* 2015). The clinical studies are in agreement with the experimental results, however the significance of the findings in these employees is difficult to predict, as the markers of oxidative stress are not specific to the effect of nanoparticles and no reference values are given. Markers of oxidative stress have been elevated in the EBC in patients with diseases caused by carcinogenic dusts silica and asbestos, comparing to control subjects (Pelclova *et al.* 2007a, 2007b, 2008). Additionally, they have been found to be overproduced in rheumatoid arthritis, atherosclerosis, and cancer (Syslova 2014, Yang 2013). Another source of nanoparticles in office workers may be the photocopiers and laser printers (Martin *et al.* 2015). To eliminate this effect in our study, we selected a control group of subjects working in the offices with otherwise similar workplace conditions. TiO_2 particles persist in the respiratory tract for several days (Pelclova *et al.* 2015); therefore the duration of biomarkers´ elevations needs to be answered by further studies.

CONCLUSIONS

This pilot study suggests that even short nanoTiO_2 exposure may lead to pulmonary oxidative stress. The results are in agreement with experimental studies and with our studies in workers producing nanoscale TiO_2 and Fe oxides. The effect may be short-term and reversible; the clinical significance is unknown, anyway there is an urgent need of further studies in humans.

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