Short Communication

Leukocyte telomere length is not affected by long-term occupational exposure to nano metal oxides

Jaroslav A. HUBACEK1*, Daniela PELCLOVA2, Dana DLOUHA1, Pavel MIKUSKA3, Stepanka DVORACKOVA4, Stepanka VLCKOVA2, Zdenka FENCLOVA2, Jakub ONDRACEK5, Martin KOSTEJN5, Jaroslav SCHWARZ5, Alex POPOV4, Kamil KRUMAL3, Vera LANSKA1, Pavel COUFALIK3, Sergei ZAKHAROV2 and Vladimir ZDIMAL5

1 Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Vídeňská 1958/9, Prague, 14021, Czech Republic
2 Department of Occupational Medicine, First Faculty of Medicine, Charles University and General University Hospital in Prague, Na Bojišti 1, Prague, 12000, Czech Republic
3 Institute of Analytical Chemistry of the CAS, v.v.i, Veveri 967/97, 60200 Brno, Czech Republic
4 Technical University in Liberec, Faculty of Mechanical Engineering, Department of Machining and Assembly, Department of Engineering Technology, Department of Material Science, Studentská 1402/2, 46117, Liberec, Czech Republic
5 Institute of Chemical Process Fundamentals CAS, v.v.i., Rozvojová 1, 16502, Prague, Czech Republic

*To whom correspondence should be addressed:
Jaroslav A. Hubacek
IKEM-CEM
Videnska 1958/9
140 21 Prague 4
Czech Republic
Tel: +420 261 363 379
Fax: +420 241 721 574
E-mail: jahb@ikem.cz

Short title: TELOMERES AND NANOPARTICLE EXPOSURE
Abstract: The aim of this study was to ascertain whether long-term occupational exposure to nanoparticles would affect relative leukocyte telomere length (LrTL). We analysed occupational exposure to size-resolved aerosol particles, with special emphasis on nanoparticles at two workshops: i/ the production of nanocomposites containing metal oxides; ii/ laboratory to test experimental exposure of nano-CuO to rodents. Thirty five exposed researchers (age 39.5 ± 12.6 years; exposure duration 6.0 ± 3.7 years) and 43 controls (40.4 ± 10.5 years) were examined. LrTL did not significantly (P = 0.14) differ between the exposed researchers (0.92 ± 0.13) and controls (0.86 ± 0.15). In addition, no significant correlation (r = -0.22, P = 0.22) was detected between the duration of occupational exposure and LrTL. The results remained non-significant after multiple adjustments for age, sex and smoking status. Our pilot results suggest that relative leukocyte telomere length is not affected by occupational exposure to nanoparticles.

Key words: Telomere length, Follow-up, Metal nanoparticles.
Although experimental data point to the deleterious health effects of exposure to engineered nanoparticles\textsuperscript{1}), the exact molecular mechanisms underlying nanotoxicity are not yet fully understood. The health consequences of occupational and environmental exposure to nanoparticles have been widely discussed. Yet, there is a distinct scarcity of data on quality exposure measurements as well as on markers of exposure and effect in the context of engineered metal oxide exposure under occupational settings. We concentrated on assessing workers with high inhalational exposure to nanoparticles. Our previous studies found increased markers of inflammation and oxidation of nucleic acids in workers exposed to nano-Fe oxides as well as nano-TiO\textsubscript{2}\textsuperscript{2-4}).

Telomeres are complexes of non-coding DNA (TTAGGG hexanucleotide tandem repeats with a total length of approximately 20,000 bp) and proteins on the ends of chromosomes in eukaryotic cells. Telomeres maintain genome stability during cell replication and play a significant role in human ageing and age-related diseases\textsuperscript{5}). Shorter telomere length leads to a decrease in the number of functional cells, which contributes to overall organ dysfunction. It is believed that telomere length within peripheral blood leukocytes reflects biological age\textsuperscript{6}) and that shorter telomeres are associated with a wide range of non-communicable diseases.

Telomere length shortens in response to unhealthy lifestyles (e.g. smoking). In this context, the aim of our pilot study was to test whether shorter telomere length might be associated with long-term exposure to occupational aerosols containing engineered metal oxide nanoparticles.

Firstly, aerosol exposure was monitored in two workshops performing the research of nanocomposites, i.e. nanoparticle producing processes such as welding, smelting and machining. The following offline and online aerosol instruments were
used: the Berner Low-Pressure Cascade impactor (Hauke, Austria) and the Condensation Particle Counters and Optical Particle Sizers P-Trak, DustTRAK DRX, SMPS 3936L and APS 3321 (all TSI, USA). Elemental analysis of size-resolved aerosol samples was performed using a Scanning Electron Microscope (SEM) equipped with Energy-Dispersive X-Ray spectroscopy (Quantax 200 with XFlash 5010 detector). The percentage of total particulate matter (PM) and number concentrations (≤10 µm in aerodynamic diameter) in larger size bins was determined by SMPS and APS. The Indusem scanning electron microscope (Tescan, UAE) was used for surface imaging.

Secondly, nanoparticle size distribution was measured online using the SMPS 3936L72 (TSI, USA) under laboratory conditions as part of inhalation experiments involving researchers working with rodents exposed to pure nano-CuO.

A total of 35 researchers occupationally exposed to nanoparticles for periods of between 2 and 20 years, were examined pre-shift on the second working day following the weekend. The basic characteristics of the groups of examined subjects are shown in Table 1.

The first group (N = 27) of researchers involved in the new nanocomposite research were exposed for an average of 6 years to nanoparticles. Production involved the following 3 processes: welding of mild steel using Metal Active Gas technology; smelting of AlSi₉Cu₃ alloys cast in bentonite moulds and melted at 830°C; grinding and machining of nanocomposite materials for surface modification. The median shift duration was 105 min (25-230).

The second group (N = 8) consisted of researchers working with rodents exposed to 100% engineered nanoparticles (including MnO•Mn₂O₃, CdO, PbO, TiO₂,
ZnO and, finally, CuO lasting three months) for an average of 5.6 ± 0.7 years at regular three-month exposure periods. The median exposure time per shift was 7 min.

Forty-three control subjects (office employees) from the same location without history of nanoparticle exposure also participated in the study.

The Ethical Committee of the First Medical Faculty, Charles University approved the study protocol. All individuals gave their signed informed consent to take part in the study. The study was conducted according to the Good Clinical Practice guidelines and agrees with the Declaration of Helsinki.

Relative telomere length was analysed in leukocyte DNA isolated from whole frozen (at -80°C for less than 4 weeks) EDTA blood. A quantitative polymerase chain reaction (qPCR)-based method described by Dlouha et al.7-9) was used. Briefly, analysis was performed in triplicate on the Rotor-Gene 3000 (Corbett Research, Ltd.) using oligonucleotides: telomere analysis 5´ GGT TTT TGA GGG TGA GGG TGA GGG TGA TTT TGA GGG TGA GGG TGA GGG TGA GGG TGA GGG T and 5´ TCC CGA CTA TCC CTA TCC CTA TCC CTA TCC CT A TCC CTA TCC CTA; single copy gene analysis 5´ CAG CAA GTG GGA A GT G TG AAT CC and 5´ CCC ATT CTA TCA TCA AGG GGT ACA A. Relative telomere length was calculated as the ratio of telomere repeats to a single-copy gene (acidic ribosomal phosphoprotein PO - 36B4).

Statistical analysis was performed by ANOVA and adjusted for sex, age and smoking status. As the mean LrTL was similar in both groups of exposed subjects and the correlation between LrTL and exposure duration in both exposed groups was of the same magnitude, they were pooled together. A P value below 0.05 was considered significant.
In the case of the two nanocomposite processing workshops, the average mass concentration in air ranged from 0.120 mg/m$^3$ during smelting to 1.840 mg/m$^3$ during welding. The highest number concentration during smelting was 2.0 x 10$^5$ #/cm$^3$, with 8.2 x 10$^5$ #/cm$^3$ for machining. The proportion of particles smaller than 100 nm in diameter ranged from 37% during welding to 97% during smelting. Chemical analysis of nano-sized fractions showed the prevalence of Fe, Mn, Si, Na, S, Cl and Al. In the case of the rodent experiment, the average mass concentration of Cu in air during exposure to nanoCuO was 7.3±3.2 ng/m$^3$. The proportion of nanoparticles was 100%.

The characteristics of the groups of researchers and controls did not significantly differ (Table 1). All examined subjects were self-reported healthy non-diabetic adults.

LrTL did not significantly (P = 0.14) differ between researchers (0.92 ± 0.13) exposed to nanocomposites and unexposed controls (0.86 ± 0.15). In exposed subjects, there was no significant correlation (r = -0.22, P = 0.22) between the duration of exposure (in total work years) to nanoparticles and LrTL. The results remained non-significant after multiple adjustments for age, sex and smoking status.

LrTL in both examined groups were comparable with the findings of our previous study of healthy adult females$^{7-8}$. The results of our study suggest that several-year-long inhalational exposure to these nanoparticles does not significantly influence leucocyte telomere length.

In the literature, human data on this topic are scarce. The studies that are available are difficult to compare as they are characterised by very heterogeneous observations on different pollutant/chemical compounds at variable exposure times. Shorter telomeres have been associated with exposure to traffic-related air pollution...
(particulate matter, benzene, toluene) or occupational exposure to polycyclic aromatic hydrocarbons, pesticides and heavy metals (in details summarised in detail by Zhang et al.\textsuperscript{10}). On the other hand, longer telomeres have been reported in subjects exposed to arsenic compounds or persistent organic pollutants\textsuperscript{10}).

Further, the number of examined subjects in some of these studies is low (less than 20); others use different methods for LrTL analysis (mostly Q-PCR, but also FISH and Southern blotting), with exposure duration varying from days to years\textsuperscript{10}).

Based on experimental studies, we know that nanomaterial toxicity may lead to cell type-dependent intracellular responses, resulting in unique disturbances in cellular function\textsuperscript{11,12}). Further, the different life spans of cells from various tissues can (1) affect potential correlations between leukocyte rTL and organ rTL\textsuperscript{8}) and (2) impact on the estimation of potential nanoparticle toxicity across different tissues. As a consequence of these factors, our pilot study could not draw any definitive conclusions.

The strength of our study, however, was the long (a mean of about 6 years) exposure time to potentially damaging metal oxide nanoparticles, which enabled us to lower the variability of the individual measurements. The study was partially limited by the fact that our exposed subjects came from two groups with different types of exposure to metal oxide nanoparticles and different shift durations. Although the average mass concentration of metal oxides was lower in the rodent laboratory, the proportion of nanoparticles was 100%. Moreover, when analysed separately, the exposed groups did not significantly differ regarding correlations between LrTL and exposure duration. In fact, as their respective LrTLs were almost identical, they were pooled together.
We thus conclude that in our group of workers exposed to aerosols containing metal oxide nanoparticles, leukocyte telomere length was not affected. This, however, does not prove their safety, nor does it exclude the potential danger of oxidation stress in workers that occupationally inhale metal oxide nanoparticles. In agreement with the WHO Guidelines on Protecting Workers from Potential Risks of Manufactured Nanomaterials (2017), more data on workers exposed to engineered nanoparticles is urgently needed if we are to find the best biomarkers of exposure.

**Conflict of Interest**

None declared.

**Acknowledgements**

The authors wish to thank all of the volunteers who took part in this study. This work was supported by the project (Ministry of Health Czech Republic) for the development of research organisation 00023001 – IKEM, Prague, Czech Republic – Institutional support; by the projects Progres Q25/LF1 and Q29/LF1 of the Charles University Prague and by project of Grant Agency of the CR P503/12/G147 and 18-02079S.
References


Table 1. Characteristics of examined subjects.

<table>
<thead>
<tr>
<th></th>
<th>Exposed</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>35</td>
<td>43</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.5 ± 12.6</td>
<td>40.4 ± 10.5</td>
</tr>
<tr>
<td>Exposure duration (years)</td>
<td>6.0 ± 3.7</td>
<td>0</td>
</tr>
<tr>
<td>Males/females (N)</td>
<td>26/9</td>
<td>28/15</td>
</tr>
<tr>
<td>Smokers (Yes/No)</td>
<td>1/34</td>
<td>3/40</td>
</tr>
<tr>
<td>Abstinence (Yes/No)</td>
<td>2/33</td>
<td>3/40</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.02 ± 5.83</td>
<td>24.72 ± 4.21</td>
</tr>
<tr>
<td>LrTL</td>
<td><strong>0.92 ± 0.13</strong></td>
<td><strong>0.86 ± 0.15</strong></td>
</tr>
</tbody>
</table>

Except for exposure duration, there were no significant differences between the parameters. Values are given as mean ± S.D.