Long-term exposures to low doses of cobalt nanoparticles induce cell-transformation enhanced by oxidative damage.

Laura Rubio: Balasubramanyam Annangi, Jordi Bach, Gerard Vales, Laura Rubio, Ricardo Marcos, Alba Hernández.

Universitat Autònoma de Barcelona, Campus Bellaterra Facultat de Biociències, Departament de Genètica i Microbiologia, Barcelona, Spain
laura.rubio@uab.cat

Abstract

A great effort is being done by the scientists to increase our knowledge on the role of nanoparticles-associated genotoxic and carcinogenic effects [1, 2]. Although important research activity took place in this area for >10 years, most of the findings concerning to the genotoxic and cell transforming potential of NPs are limited to short-term in vitro studies [3, 4]. Comprehensively, acute or short-term studies use high environmentally irrelevant single doses to study the adverse effects of engineered NPs, which could not be enough to draw plausible conclusions about the potential human health risk of NPs exposure [5]. Until now, only a few in vitro long-term exposure studies have been carried out with NPs [6,7,8,9]. Keeping this in mind, it seems necessary to increase the amount of in vitro investigations focusing on long-term or chronic exposures at sub-toxic doses.

Despite the usefulness of cobalt nanoparticles (CoNPs) in various fields [10], they are also potentially harmful to humans. Some studies have found that in vitro acute exposure of CoNPs induce oxidative stress, DNA damage, morphological transformation and inflammatory responses in different cell types, among other kind of effects [3, 11, 12, 13, 14,15]. However, there is no conclusive information available on the in vitro carcinogenic potential of CoNPs under chronic settings so far. In vitro cell transformation assays have been proposed as alternatives to long term animal studies. In fact, OECD has specific guidelines on ‘‘Cell transformation assays for detection of chemical carcinogens’’ [16] with accumulated evidence that the cellular and molecular processes involved in vitro cell transformation are similar to those of in vivo carcinogenesis [17].

In this study, we have evaluated the cell transforming ability of cobalt nanoparticles (CoNPs) after long-term exposures (12 weeks) to sub-toxic doses (0.05 and 0.1 μg/mL). To get further information on whether CoNPs-induced oxidative DNA damage is relevant for CoNPs carcinogenesis, the cell lines selected for the study were the *wild-type* mouse embryonic fibroblast (MEF *Ogg1*$^{+/+}$) and its isogenic *Ogg1 knockout* partner (MEF *Ogg1*$^{-/-}$), unable to properly eliminate the 8-OH-dG lesions from DNA. Our initial short-term exposure experiments demonstrate that low doses of CoNPs are able to induce reactive oxygen species (ROS) and that MEF *Ogg1*$^{-/-}$ cells are more sensitive to CoNPs-induced acute toxicity and oxidative DNA damage. On the other hand, long-term exposures of MEF cells to sub-toxic doses of CoNPs were able to induce cell transformation, as indicated by the observed morphological cell changes, significant increases in the secretion of metalloproteinases (MMPs) and anchorage-independent cell growth ability, all cancer-like phenotypic hallmarks. Interestingly, such changes were significantly dependent on the cell line used, the *Ogg1*$^{-/-}$ cells being particularly sensitive. Altogether, the data presented here confirms the potential carcinogenic risk of CoNPs and points out the relevance of ROS and *Ogg1* genetic background on CoNPs-associated effects.

References

[1] Arora S, Rajwade JM, Paknikar KM. Toxicol Appl Pharmacol. **258**(2012):151–65.

[2] Becker H, Herzberg F, Schulte A, Kolossa-Gehring M. Int J Hyg Environ Health **214**(2011):231–8.

[3] Colognato R, Bonelli A, Ponti J, Farina M, Bergamaschi E, Sabbioni E, et al. Mutagenesis **23**(2008):377–82.

[4] Ponti J, Broggi F, Mariani V, De Marzi L, Colognato R, Marmorato P, et al. Nanotoxicology **7**(2013):221–33.

[5] Hristozov DR, Gottardo S, Critto A, Marcomini A. Nanotoxicology **6**(2012):880–98.

[6] Hackenberg S, Scherzed A, Technau A, Froelich K, Hagen R, Kleinsasser N. J Biomed Nanotechnol **9**(2013):86–95.

[7] Huang S, Chueh PJ, Lin YW, Shih TS, Chuang SM. Toxicol Appl Pharmacol **241**(2009):182–94.

[8] Jacobsen NR, Saber AT, White P, Møller P, Pojana G, Vogel U, et al. Environ Mol Mutagen **48**(2007):451–61.

[9] Kocbek P, Teskac K, Kreft ME, Kristl J. Small **6**(2010):1908–17.

[10] Horev-Azaria L, Kirkpatrick CJ, Korenstein R, Marche PN, Maimon O, Ponti J, et al. Toxicol Sci **122**(2011):489–501.

[11] Magaye R, Zhao J, Bowman L, Ding M. Exp Ther Med **4**(2012):551–61.

[12] Papageorgiou I, Brown C, Schins R, Singh S, Newson R, Davis S, et al. Biomaterials **28**(2007): 2946–58.

[13] Papis E, Gornati R, Prati M, Ponti J, Sabbioni E, Bernardini G. 2007. Toxicol Lett **170**(2007):185–92.

[14] Petrarca C, Perrone A, Verna N, Verginelli F, Ponti J, Sabbioni E, et al. Int J Immunopathol Pharmacol **19**(2006):11–14.

[15] Ponti J, Broggi F, Mariani V, De Marzi L, Colognato R, Marmorato P, et al. Nanotoxicology **7**(2013):221–33.

[16] Vasseur P, Lasne C. Mutat Res **744**(2012):8–11.

[17] Combes R, Balls M, Curren R, Fischbach M, Fusenig N, Kirkland D, et al. ATLA **27**(1989):745–67.