Involvement of oxidative stress and calcium signaling in oxide nickel nanoparticles - induced alterations in human pulmonary artery endothelial cells

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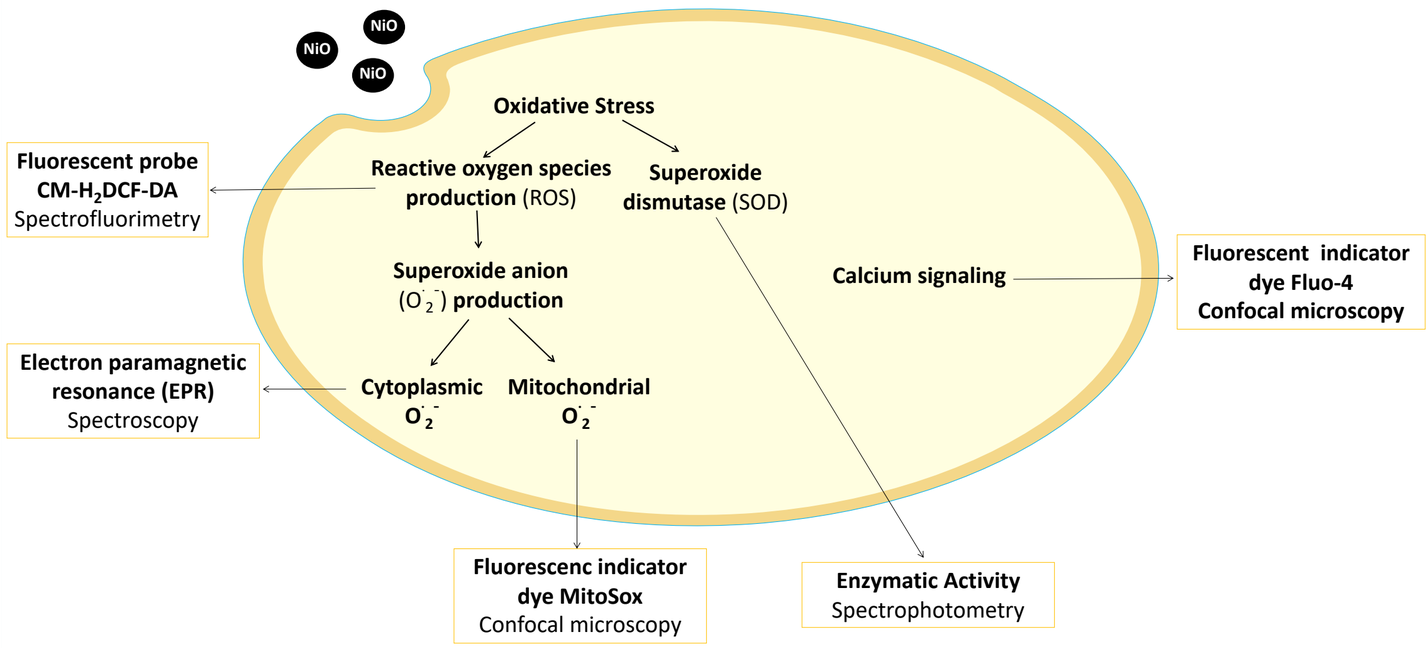
Objectives

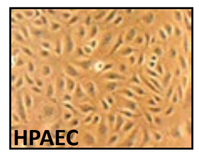
Anthropic activities such as mining amplifies the natural erosion of nickel mines leading to atmospheric emission of oxide nickel nanoparticles (NiONPs). New Caledonia is particularly affected by nickel mining activities. After inhalation, NPs penetrate deeply into the airways and exert deleterious effects on cardiovascular system. Pulmonary artery endothelial cells (HPAEC) can be a direct target of inhaled particles. Alteration in oxidative stress and calcium homeostasis are critical events involved in the physiopathology of vascular diseases such as pulmonary hypertension. Only a few studies have investigated the effect of NiONPs on pulmonary vascular endothelial cells and the cellular mechanisms remain unclear. The aim of this study was to assess the cytotoxic effects of NiONPs on HPAEC, especially oxidative stress and calcium signaling. HPAEC were exposed for 4 or 24 h to NiONPs (0.5 to 150 µg/cm²). Different endpoints were studied (i) ROS production (ii) cytoplasmic and mitochondrial superoxide anion production (iii) superoxide dismutase activity and (vi) calcium signaling.

Materials and Methods



Cells :
HPAEC : Human Pulmonary Artery Endothelial Cells

Particles :
NiONPs (Nickel(II) oxyde nanoparticles)



Results

Cellular ROS production (CM-H_2DCF-DA)

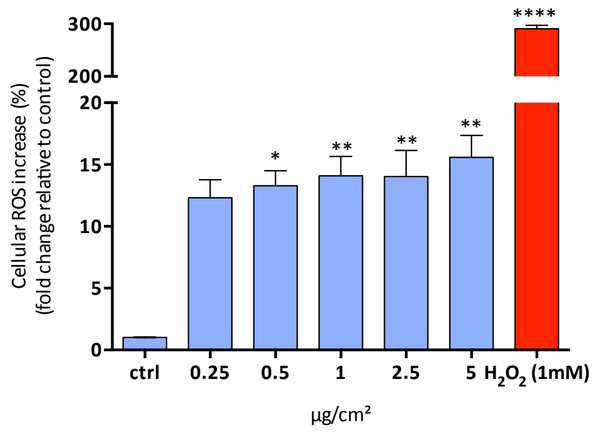


Fig 1 : Intracellular global ROS production in HPAEC.
After a 4h-exposure to NiONPs (0.25 – 5 µg/cm²) significantly increase from 0.5 µg/cm². The values were normalized to untreated cells. Data are Mean ± SEM of four independents experiments (n=4), in triplicate. Statistically significant at $P < 0.05$(*), $P < 0.01$(**), and $P < 0.001$(***).

Cytoplasmic $O_2^{\cdot -}$ production (EPR)

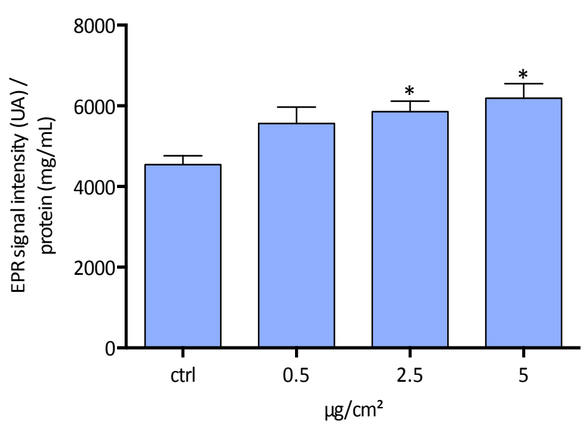


Fig 2 : Cytoplasmic $O_2^{\cdot -}$ production in HPAEC.
After a 4h-exposure to NiONPs (0.5 – 5 µg/cm²) significantly increase from 2.5 µg/cm² in a concentration-dependant manner. Data are Mean ± SEM of three independents experiments (n=3), in triplicate, statistically significant at $P < 0.05$(*). EPR intensity is expressed in unit intensity in unit intensity/ mg/mL of proteins (measured by Lowry test).

Mitochondrial $O_2^{\cdot -}$ production (MitoSox)

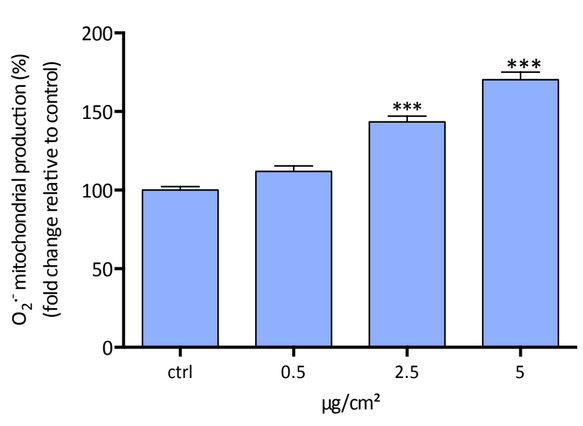


Fig 3 : Mitochondrial $O_2^{\cdot -}$ production in HPAEC.
After a 4h-exposure to NiONPs (0.5 – 5 µg/cm²) significantly increase from 2.5 µg/cm² in a concentration-dependant manner. Data are Mean ± SEM of three independents experiments (n=3), in triplicate, statistically significant at $P < 0.001$(***).

SOD activity (Spectrophotometry)

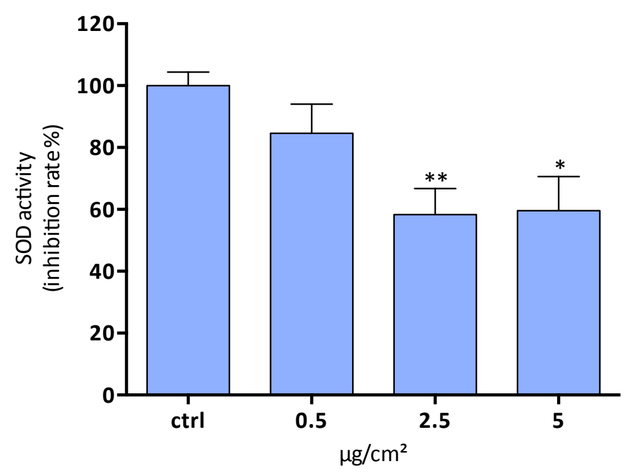


Fig 4 : SOD activity in HPAEC.
After a 24-h exposure to NiONPs exposure (0.5 – 5 µg/cm²) significantly decrease from 2.5 µg/cm². Data were mean ± SEM of three independents experiments, (n=3), in triplicate. Statistically significant at $P < 0.05$(*) and $P < 0.01$(**), as compared to untreated cells.

Intracellular Ca^{2+} (Fluo-4AM)

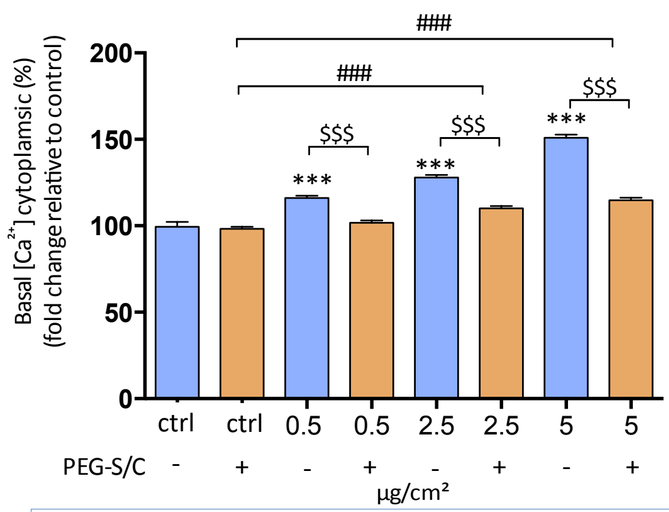


Fig 5 : Cytoplasmic basal calcium level ($[Ca2+]_c$) in HPAEC.
Calcium level measured by confocal microscopy. with Fluo-4AM probe (1 µM). After a 4h-exposure to NiONPs (0.5 - 5 µg/cm²), intracellular calcium ion levels $[Ca^{2+}]_i$ significantly increase. A 1h-pretreatment with PEG–SOD (600 U/mL) and PEG–CAT (300 U/mL) significantly decrease NiONPs - induced $[Ca^{2+}]_i$ alterations, suggested the role of oxidative stress in calcium homeostasis impairment. Data were mean ± SEM of three independents experiments, (n=3), in triplicate. Statistically significant at $P < 0.001$(***) as compared to control without PEG-S/C ; $P < 0.001$(###), as compared to untreated cells with PEG-S/C.

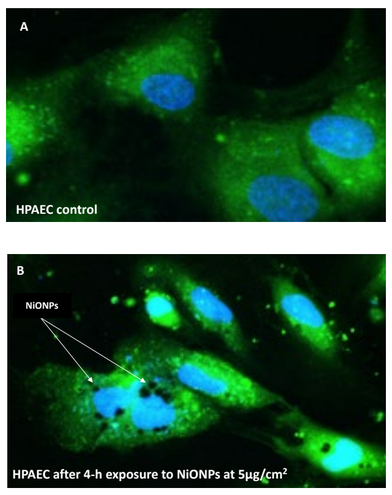


Fig 6 : HPAEC imaging of Fluo-4AM probe (1µM) by confocal microscopy.
After a 4h-exposure with NiO NPs at 5 µg/cm²
(A) control, (B) NiO NPs treated cells

Conclusion

Our results seem to show that NiO NPs-induced intracellular calcium homeostasis impairment is closely correlated to oxidative stress and thus could present a cardiovascular disease risk.