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Effect of UFP produced by anthropogenic combustion on human lung macrophages and motor neurons: role in pulmonary inflammation and neurodegeneration

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Anthropogenic particulate matter (PM) is one of the primary pollutants found in the atmosphere of industrialized cities. It can be inhaled by humans and accumulate in the lungs. According to recent research, PM is harmful to respiratory conditions. The primary cause of these effects is the ultrafine particles (UFP, PM < 100 nm), which are effectively deposited in the alveolar, tracheobronchial, and nasal regions due to their PM size. However, after air pollution inhalation, UFP has a strong ability to penetrate human brain deeply, with a poor possibility to be cleared. In this work, we examined how UFP affected the activation of human lung macrophages (HLMs) mainly involved in the inflammatory response to injury and neuronal function by measuring the release of proinflammatory cytokines and chemokines, the generation of reactive oxygen species (ROS), and the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in macrophages, and organellar dysfunction and neurotoxicity in motor neurons and primary cortical neurons. Additionally, a well-designed analytical procedure, set-up in a previous paper [1], allowed UFP to be fractionated in nanoparticles larger (NP100) and smaller (NP20) than 20 nm after the organic fraction was removed, allowing for the isolation of each particle's unique contribution. More in detail, dichloromethane (DCM) was used to extract UPFin order to separate the particles from the organic carbon soluble in DCM. Additionally, dry particles were dissolved in N-methyl-pyrrolidinone (NMP). The soot particles with a diameter more than 20 nm were separated from the NMP dispersions using membrane filters (Anodisc).

It's interesting to note that NP100 had no effect on the release of HLM cytokines, whereas PM0.1 and NP20 did. Specifically, PM0.1 caused HLMs to release IL-6, IL-1β, and TNF-α but not CXCL8. Furthermore, HLMs' preformed mediator β-glucuronidase release was not induced by UFP, NP20, or NP100. Given the lengthy (18 h) period required for the release of cytokines, it is possible that UFP and NP20 will cause the tested mediators to be produced ex novo.Consequently, mRNA expression of IL-6, TNF-α, and IL-1β was induced by UFP and NP20 after 6 hours of incubation. Furthermore, without causing cytotoxicity, NP20 induced the production of ROS and an increase in $[Ca^{2+}]_i$ in a time-dependent manner.

Furthermore, PM0.1 and NP20 exposure induced detrimental effects in motor neurons through the dysfunction of mitochondrial and endoplasmic reticulum (ER), the main calcium storing organelles deputed to relevant cellular functions such as energetic metabolism and protein folding. In this respect, after 48 hrs of incubation, PM0.1 and NP20induced ER stress, a type of apoptotic cell death measured as

organellar calcium dysfunction, and BIP and CHOP protein expression. Of note ER stress induced by PM0.1 and NP20 in motor neurons was associated to pathological changes in ER morphology and dramatic reduction of organellar Ca^{2+} level through the dysregulation of the Ca^{2+}-pumps SERCA2 and SERCA3, the Ca^{2+}-sensor STIM1, and the Ca^{2+}-release channels RyR3 and IP3R3.

An exemplificative scheme of the role of UFP role in pulmonary inflammation and neurodegeneration is reported in Figure 1.

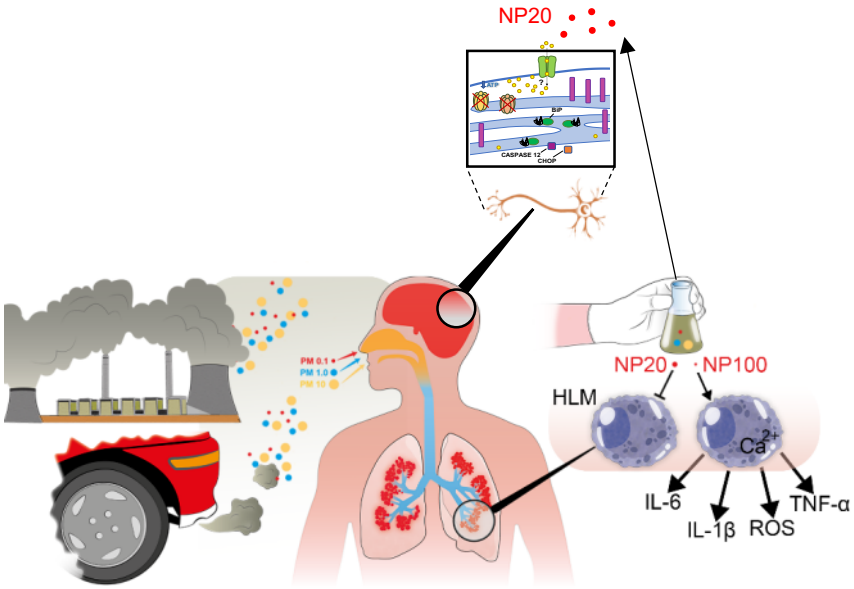

Figure 1- Scheme of the role of UFP role in pulmonary inflammation and neurodegeneration.

All of the current data point to NP20's primary detrimental effect among PM fractions [2]. This is especially concerning because the existing analytical techniques do not readily measure this fraction at the exhausts, making it impossible to set legal limits. This means that new monitoring methods and strategies to limit NP20 formation must be found.

[1] Marcella S., Apicella B., Secondo A., Palestra F., Opromolla G., Ciardi R., Tedeschi V., Ferrara A. L., Russo C., Galdiero M. R., Cristinziano L., Modestino L., Spadaro G., Fiorelli A., Loffredo S., *Environment International*, **2022,** 166, 107395.

[2] Sapienza, S.; Tedeschi, V.; Apicella, B.; Palestra, F.; Russo, C.; Piccialli, I.; Pannaccione, A.; Loffredo, S.; Secondo, A. *Int. J. Mol. Sci.,***2022** 23, 13041.