Boron nitride nanosheets can trigger lipid-mediated autophagy in lung alveolar epithelial cells

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Hexagonal boron nitride (*h*-BN) is a newly introduced 2D material gaining wider applications in different sectors from electronics and textiles to biomedical and therapeutics. However, there is a limited understanding of their interaction with lung cells and potential consequences thereafter including the stress response mechanism.

In this study, we investigated the localization of *h*-BN nanosheets at cellular and sub-cellular levels (i.e., in lysosomes) after exposure to alveolar lung epithelial cells (A549 cell line) cultured under air–liquid interface conditions.

Our results showed a significant uptake of *h*-BN nanosheets in cells with a partial co-localization in lysosomes as determined by applying a range of analytical and high-resolution imaging approaches such as flow cytometry, ICP-MS, confocal laser scanning microscopy, RAMAN-confocal microscopy, and STEM-EDX. There was no significant ($p>0.05$) acute toxicity (LDH release), loss of epithelial barrier integrity (TEER), or oxidative damage observed in cells at tested dose ranges (1, 5, and 10 µg/cm^2) for 24 h. However, we observed an imbalance in lipid metabolisms as higher deposits of lipid granules were observed in cells after exposure to *h*-BN as compared to control. To understand further the downstream effect of lipid accumulation in cellular stress, we investigated whether the autophagy pathway was activated. Interestingly, we observed a significant ($p<0.05$) induction of autophagy in cells after exposure to *h*-BN as evidenced by autophagic assay. The immunofluorescence imaging further confirmed enhanced accumulation of LAMP-1 and LC3-B positive vacuoles in *h*-BN exposed cells as compared to control. The observed effects on autophagy could potentially be associated with the downstream processing and breakdown of excess lipid granules to maintain lipid homeostasis. Indeed, we observed lysosomal co-localization of lipid granules supporting this argument. In addition, once the autophagy induction was blocked using wortmannin (a known PI3K inhibitor), the *h*-BN exposure showed a slight improvement in cell death confirming a protective role of autophagy.

Overall, our results suggest that continuous exposure to *h*-BN for the long term may pose autophagic arrest due to insufficiency of autophagic flux that may consequently provoke adverse outcomes (i.e., metabolic disorders or immune diseases) potentially due to imbalanced lipid accumulation in the lungs.

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