Respiratory Physiology

Nanoparticle Fate in Alveolar Epithelial Cells: Uptake, Distribution and Exocytosis

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Introduction. Strategies for enhancement of nanoparticle-driven AEC gene/drug delivery and/or amelioration of AEC nanoparticle-related cellular toxicity is an important target in the next future.

Objective: To prove that autophagic processing of nanoparticular is essencial for maintenance of alveolar epithelial cells.

Material and Methods: Utilizing confocal microscopy, we quantitatively assessed uptake, processing and egress of near infrared (NIR) fluorescence-labeled polystyrene nanoparticles (PNP) in live primary rat alveolar epithelial cell (AEC) monolayers (RAECM) after apical exposure. Intracellular PNP content was assessed by measuring the time courses of NIR fluorescence intensity for PNP of 20, 100 and 200 nm and for apical [PNP] (20 nm) of 40, 80 and 160 ng/mL. PNP content and colocalization with intracellular vesicles (including autophagosomes, lysosomes, Golgi and endoplasmic reticulum) in PNP-exposed AEC were determined over the entire cell volume via z-stacking. Involvement of endocytosis in PNP uptake was tested by pharmacologic inhibition of classical endocytotic pathways. Colocalization of early endosome marker Rab5-GFP with PNP in apically exposed AEC was investigated for up to 3 hr. The role of autophagy in intracellular processing of PNP or ambient pollution particles (APP, diameter ≤200 nm) was assessed using LC3 (microtubule-associated protein 1A/1B light chain 3B)-II immunolabeling and inhibitors of autophagosome formation (3-methyladenine (3-MA)) or autolysosome formation (bafilomycin or chloroquine). Mechanisms of PNP uptake into and egress from RAECM were further studied by inhibition of microtubule polymerization required for movement of PNP-filled intracellular vesicles (e.g., autophagosomes) and mobilization of intracellular [Ca^{2+}] known to speed up exocytosis. Isotropic cuvette-based microfluorimetry was used to

determine intracellular [PNP] from PNP content assessed by anisotropic confocal microscopy.

Results: Uptake rates and steady state intracellular content decreased as PNP size increased from 20 to 200 nm. Uptake rates and steady state intracellular content increased with increased apical [PNP] (20 nm) and were unaffected by inhibition of endocytic pathways. <3% of total intracellular PNP colocalized with Rab5 positive intracellular vesicles post-PNP exposure for up to 3 hr. Both PNP and APP exposure led to marked increases in LC3-II expression, and PNP increasingly co-localized with autophagosomes and/or lysosomes over time. PNP egress exhibited both fast [Ca^{2+}]-dependent release and a slower diffusion-like process. Inhibition of microtubule polymerization curtailed initial rapid PNP egress, causing elevated vesicular and intracellular PNP content. Interference with autophagosome formation led to slower PNP uptake and markedly decreased steady state intracellular PNP content. At steady state, cytosolic [PNP] was higher than apical [PNP], and vesicular [PNP] (~80% of intracellular PNP content) exceeded both cytosolic [PNP] and intracellular [PNP].

Conclusions: These findings are consistent with the hypotheses that (1) autophagic processing of nanoparticles is essential for maintenance of AEC integrity, (2) altered autophagy and/or lysosomal exocytosis may lead to AEC injury and (3) intracellular [PNP] in AEC is regulable, suggesting strategies for enhancement of nanoparticle-driven AEC gene/drug delivery and/or amelioration of AEC nanoparticle-related cellular toxicity.

Keywords: polystyrene nanoparticle, alveolar epithelial cells, lysosomal exocytosis