The Efficiency of Cytosolic Drug Delivery using pH-responsive Endosomolytic Polymers does not Correlate with Activation of the NLRP3 Inflammasome

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Statement of Purpose: Inefficient cytosolic delivery has limited the development of many promising biomacromolecular drugs, a long-standing challenge that has prompted extensive development of drug carriers that facilitate endosomal escape.[1] Although many such carriers have shown considerable promise for cytosolic delivery of a diversity of therapeutics, the rupture or destabilization of endo/lysosomal membranes has also been associated with activation of the NLRP3 inflammasome.[2] When activated, the inflammasome causes downstream secretion of IL-1β and IL-18, leading to widespread inflammation.[2] In this study, we investigated relationships between pH-dependent membrane destabilization, cytosolic drug delivery, and inflammasome activation using a series of well-defined poly[(ethylene glycol)-block-[(2-(dimethyl amino)ethyl methacrylate)-co-(butyl methacrylate)] (PEG-*b*-(DMAEMA-co-BMA)) copolymers of variable second block composition and pH-responsive properties.[3]

Methods: The polymer series was synthesized using RAFT polymerization. Endosomal escape abilities of the polymers were quantified using an erythrocyte hemolysis assay, in which lysis of red blood cells is used as a surrogate for endosomal membrane destabilization. Lysosomal rupture was quantified by co-incubating THP1 cells, polymer, and acridine orange (a lysosome accumulating dye), and then measuring acridine orange release (corresponding to lysosomal rupture) with flow cytometry. Inflammasome activation was quantified by measuring IL-1β secretion from THP1 cells and THP1 cells deficient in the inflammasome (THP1defNLRP3). After treating with polymers, IL-1β in the supernatant was measured with an ELISA. To evaluate cytosolic delivery, polymers were complexed with luciferase siRNA and used to treat luciferase expressing MDA-MB-231 cells. Amount of luciferase knockdown, a surrogate for cytosolic delivery, was quantified using quantitative luminescent imaging.

Results: A series of PEG-*b*-(DMAEMA-co-BMA) diblock copolymers were successfully synthesized, where the amount of BMA in the hydrophobic block was varied from 0% BMA to 100% BMA to create a series containing six polymers of variable second block composition, namely 0, 30, 40, 50, 60, and 100% BMA, referred to henceforth as 0B, 30B, 40B, 50B, and 100B. The erythrocyte hemolysis assay showed that 50B and 60B were the most endosomolytic (Fig 1A), whereas the lysosomal rupture assay showed that 0B, 30B, and 40B caused the most lysosomal rupture (Fig 1B). The IL-1β secretion assay showed that 0B, 30B, and 40B caused the most inflammasome activation (Fig 1C), which corresponds to the polymers that cause lysosomal rupture. Lastly, the luciferase siRNA delivery assay showed that 50B was the best at cytosolic delivery, followed by 60B (Fig 1D), which were the most endosomolytic polymers.

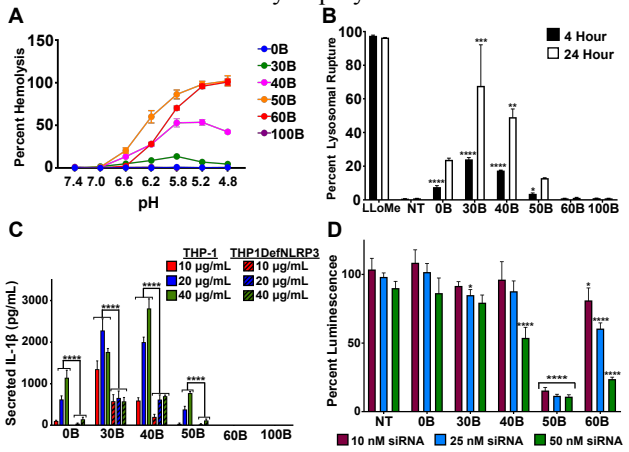

Fig 1. (A) Endosomal escape ability of the polymers. (B) Lysosomal rupture capabilities of polymers. (C) Inflammasome activation of polymers. (D) Cytosolic delivery capabilities of polymers.

Conclusions: Our results indicate that potent endosomal escape activity does not obligate, or directly correlate with, activation of the NLRP3 inflammasome. Instead, polymer-mediated lysosomal rupture was more predictive of inflammasome activation. We hypothesize that carriers lacking sufficient BMA content to escape early endosomes instead traffic to lysosomes where they mediate lysosomal rupture or membrane damage, potentially via a proton sponge mechanism mediated by DMAEMA groups (Fig 2). In most cases, inflammasome activation should be minimized as it can lead to undesirable inflammatory responses; however, in some cases this may be desirable, for example, in vaccine delivery. These studies reveal the importance of establishing relationships between polymer properties and inflammasome signaling to better design biomaterials for specific applications.

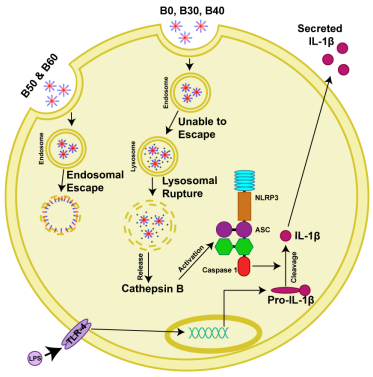

Fig 2. Schematic of hypothesis. B0, B30, and B40 causes lysosomal rupture which leads to inflammasome activation.

References:
[1] Pack DW. Nat Rev Drug Discov. 2005;4:581-593.
[2] Guo, HT. Nat Med. 2015;21:677-687.
[3] Nelson, CE. ACS Nano. 2013;7:8870-80.