Sustained Suppression of Mitochondrial Oxidative Stress using Curcumin conjugated Poly (β- Amino Esters) Nanogels.

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Statement of Purpose: Mitochondria are considered to be the energy power plants of the cell, but can also initiate and execute cell death, stimulated by oxidative stress (OS) conditions due to increased reactive oxygen species levels(ROS) (Orrenius, Gogvadze et al. 2007) . OS induced mitochondrial dysfunction is characterized by a loss in oxygen consumption and reduced ATP production, and it has been linked to a wide variety of diabetic, cardiovascular, and neurodegenerative disorders. Curcumin is considered as a potential drug to suppress mitochondrial oxidative stress but fast metabolism and aqueous insolubility prevent it from being an effective therapeutic. A lot of work has been done to incorporate curcumin into liposomal/micellar nanocarriers for improving the delivery of its active form. But most formulations have limited % weight loading, limited control over the size, and exhibit significant burst release of curcumin. To resolve these problems, we have utilized poly (β-amino ester) (PBAE) chemistry to synthesize curcumin PBAE (C-PBAE) nanogels via self-precipitation technique in dilute conditions. PBAEs are hydrolytically bio-degradable polymeric systems viable for biomedical applications (Hawkins, Milbrandt et al. 2011). Upon hydrolytic degradation of the ester bond, these nanogels show uniform release of active curcumin over 24 hours and, upon induced oxidative stress injury using H_2O_2 , have demonstrated prevention of mitochondrial oxidative stress thereby restoration of cellular bioenergetics.

Methods: The alcohol groups of curcumin were converted into acrylate to form curcumin diacrylate (CDA). Curcumin conjugated PBAE nanogels are synthesized by reacting CDA with a primary diamine (4 reactive hydrogens) in dilute conditions with ratio of 1:1. The solution was centrifuged and washed 3 times in THF. Hydrodynamic radius and morphology of these nanogels were analyzed using DLS and SEM. Degradation and curcumin release profile was analyzed by incubating the nanogels in 0.1%SDS-PBS buffer at 37°C using HPLC and UV-Vis, and antioxidant activity of released curcumin was analyzed using TEAC assay. Cell toxicity due to curcumin nanogels were analyzed on human umbilical vein endothelial cells (HUVECs) using Calcein AM red-orange as a live cell tracer. Cells were exposed to nanogels/free curcumin treatment for 0 to 24 hours followed by 0.25mM hydrogen peroxide for 2 hours as an oxidative stress inducer. Seahorse XF96 analyzer was used to analyze real time mitochondrial response towards mito stress assay developed by Seahorse Biosciences.

Results: With 60 weight % drug loading w.r.t. total nanogel mass, SEM analysis demonstrated spherical shaped nanogels with poly dispersity of +/- 20nm while hydrodynamic diameter analysis using DLS showed the size compliance with SEM results. Notably, particle size was tunable from 60 to 400 nm by varying the feed reactant concentrations. Based upon UV-Vis analysis of hydrolytically degraded products, release profile of over 24-36 hours was observed with no burst curcumin release while trolox equivalent activity assay showed the sustained anti-oxidant activity of released curcumin. Cell viability assay showed no significant cytotoxicity up to 70μg/ml of equivalent loaded curcumin while pure curcumin had IC50 value of 7.5μg/ml only. Seahorse XF mito stress kit analysis of nanogel (5μg/ml) treatment exhibited protection against H_2O_2 induced oxidative stress even after 24 hours by maintaining oxygen consumption rate, ATP production. On the other hand, curcumin (1μg/ml) though benign towards cells did not show an effective protection at any time while 5μg/ml shut the mitochondrial function altogether.

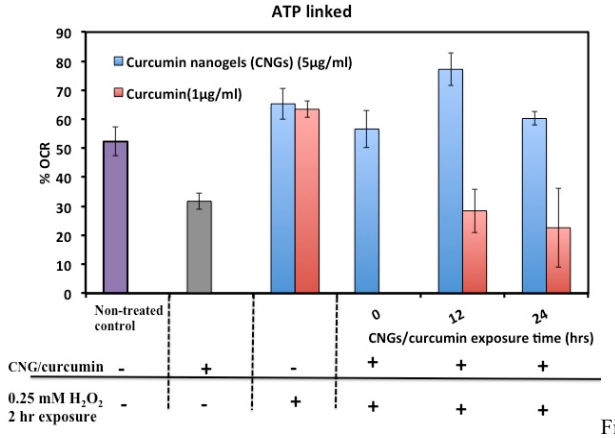

Figure 1. ATP linked oxygen consumption rate (OCR) after HUVECs exposure to curcumin nanogels (CNGs)/curcumin followed by 2 hour treatment with H_2O_2

Conclusions: Utilizing the michael addition chemistry of PBAEs, self-precipitated C-PBAE nanogels in dilute reaction conditions were synthesized with control over the size. These curcumin PBAE pro-drug nanogels greatly enhanced the release rates, controlled the initial burst release, and stabilized highly labile drugs for an extended period of time. Real time response analysis of mitochondrion bioenergetics showed continuous prolonged protection against mitochondrial oxidative stress due to uniform sustained release of active curcumin from pro-drug C-PBAE nanogel.

References: Hawkins, A. M., T. A. Milbrandt, D. A. Puleo and J. Z. Hilt (2011). "Synthesis and analysis of degradation, mechanical and toxicity properties of poly(beta-amino ester) degradable hydrogels." Acta Biomater **7**(5): 1956-1964.

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