Implications Towards Targeted Delivery of Pentagalloyl Glucose for Regenerating Elastin in Emphysematous Lungs

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Statement of Purpose: Pulmonary emphysema is one of the two pathological conditions encompassed by the term Chronic Obstructive Pulmonary Disease (COPD). COPD is mainly caused by cigarette smoking with a death toll of 18 million Americans [1]. It has been established that destruction of elastin in the alveolar walls is one of the major mechanisms involved in the progression of the emphysema [2]. Pentagalloyl glucose (PGG), a derivative of tannic acid (TA) has been shown to protect matrix elastin from elastase activity and also aid in elastin deposition in the extracellular matrix (ECM) [3]. Here we tested if elastin deposition of rat pulmonary fibroblasts can be increased by the use of PGG either in normal conditions or under inflammatory conditions (addition of TNF-α).

Methods: In vitro cell culture studies: Primary rat pulmonary fibroblasts (Cell Biologics, IL, USA) were grown in Dulbecco's Modified Eagle Medium (DMEM), (ScienCell, CA, USA). Cells were divided into four groups (n=3 per group) namely control (DMEM only), PGG treatment (10 µg/mL), tumor necrosis factor (TNF-α) treated (Peprotech Inc.) (50 ng/ml), TNF-α (50 ng/mL) combined with PGG (10 µg/mL) treatment. On days 14 and 21 cells were lysed and pellet containing ECM elastin was obtained by centrifugation. After boiling this pellet in 0.25M oxalic acid insoluble elastin was quantified using Fastin kit (Biocolor®, UK). Values obtained were normalized to the intracellular protein content and *p*-values less than 0.05 were considered as being significant. Identical groups were also grown for imaging ECM elastin using FITC anti-elastin antibody (Bioss, MA, USA). Cell viability was calculated using LDH assay. Active matrix metalloproteinase (MMP-2 and MMP-9) activity was analyzed in the spent medium, collected from cell cultures at specific time points, by gelatin zymography, described in [4]. Density of clear bands was analyzed using GelQuant software and reported as relative density units when compared to that of control cells.

In vivo targeting of NPs: Six week old male Sprague-Dawley (SD) rats (n=6) were divided into two groups which received 250U/kg of Porcine Pancreatic Elastase (PPE) (Elastin Products Co. Inc., MI, USA) dissolved in 200 µl of saline or only saline. Four weeks of time was allowed for the emphysema to develop. DiR dye (Biotium Inc., CA, USA) loaded bovine serum albumin (BSA) (SeraCare, MA, USA) nanoparticles were prepared and coated with rabbit anti-elastin (US Biological, MA, USA) antibody to target damaged elastin in lungs. The procedure used was originally described by Lei et al [5]. At the end of four weeks nanoparticles were injected through tail vein of rats and one day after injection the rats were sacrificed. Lung compliance was measured post-mortem and organs were harvested for imaging of nanoparticles and histology.

Results: At day 21 TNF-α+PGG and PGG treated groups had significantly higher elastin than the control group and TNF-α group (266.1±99.9 and 269.69±73.3 vs 127.56±22.7 and 266.1±99.9 and 269.69±73.3 vs 103.3±59 µg elastin/ mg intracellular protein respectively) (Fig 1). Immunofluorescence studies showed PGG treated group cell cultures had organized fibrous elastin deposition. Cell viability did not change with the addition of PGG to the cell cultures. Gelatin zymography showed an increase in MMP-2 and MMP-9 activity in the spent medium samples of TNF-α treated group and addition of PGG mitigated this in TNF-α+PGG samples significantly. An increase in lung compliance was observed for elastase treated group and histology showed increased alveolar spaces and loss of elastin fibers in the connective tissue of elastase treated rats compared to control rats. IVIS® Lumina XRMS (PerkinElmer, MA, USA) imaging allowed us to see nanoparticle targeting of damaged elastin networks of elastase treated rats' lungs while control group showed no such targeting.

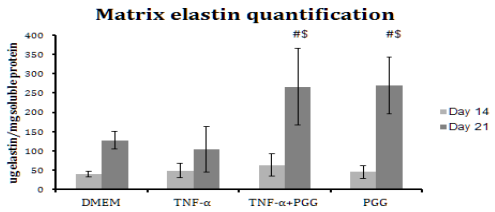

Fig.1 Bar chart showing matrix elastin quantified using FASTIN protocol. $ indicates significantly different from control group. # indicates significantly different from TNF-α group.

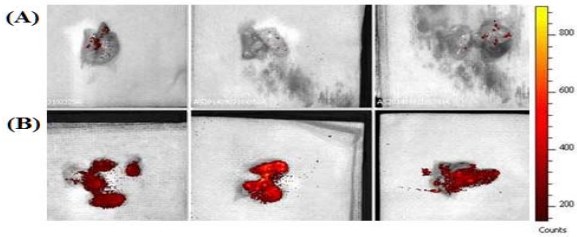

Fig.2 Nanoparticles targeting in rats' lungs. A-control rats; B- elastase treated rats.

Conclusions: We demonstrate that PGG treated cells not only show increased matrix elastin content but also it is organized in the fiber form. We also show specific targeting of damaged elastin networks in the lungs of elastase treated rats. Based on this preliminary study we hope to develop a promising PGG treatment strategy for emphysema, which requires the restoration of alveolar elastin fiber matrix.

References:

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