Ain Shams University.
Faculty of women for Arts,
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Zoology Department.

Nanostructured therapy against bleomycin induced lung fibrosis in male C57BL/6

Submitted
By

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(**Physiology**)

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APPROVAL SHEET

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CONTENTS

ABSTRACT

Pulmonary fibrosis is a chronic, progressive inflammatory lung disorder with well-established histopathological and pulmonary architectural changes. It is usually associated with significant impairment of respiratory functions Indeed, inflammatory lung disorders are eventually associated with loss of alveolar architecture, accumulation of myofibroblasts, extensive extracellular matrix deposition and remodeling of lung parenchyma.

This study aims to evaluate the therapeutic effectiveness of loading dexamethasone on Nano chitosan particles as a novel Nano-targeted treatment for bleomycin-induced pulmonary fibrosis in male C57BL/6 mice model. This is achieved with minimal systemic and cellular side effects and effective drug release of dexamethasone to the lung tissue. Dexamethasone loaded on Nano chitosan treatment was further compared with the widely used treatment dexamethasone either alone or combined with the improved form of curcumin which is nano curcumin.

Nano chitosan (NCH) particles were chemically analyzed by scaning electron microscope (SEM) Transmission electron microscope (TEM), Energy dispersive X-ray analysis (EDXA) and Fourier transform infrared spectroscopy (FTIR). These chemical analysis were carried out for Nano-chitosan particles before and after loading with dexamethasone. Chemical characterization of the novel synthesized nano particle

confirmed that dexamethasone was effectively loaded onto NCH-particles.

In vivo experiment was achieved to attain this goal on a total number of 128 male C57BL/6 mice with average weight 18-33 gm. They were divided into eight groups each of 16 mice. The first group served as normal control group which received distilled water, the second group represented the dexamethasone group which was injected intraperitoneally with a dose of 0.45 mg/kg body weight/ day for 4 weeks. The third group represented the Nano-curcumin group which was given a dose of 100 mg/kg body weight of nanocurcumin /day for 4 weeks. The fourth group represented the bleomycin group which was given a single intra-tracheal dose of BLM 1 mg/ kg body weight. The fifth group represented the bleomycin + dexamethasone. The sixth group represented the bleomycin + dexamethasone loaded on Nano chitosan particles. The seventh group represented the bleomycin + Nano-curcumin and the eighth group represented the bleomycin + dexamethasone + Nano-curcumin. Mice were dissected after two durations of 14 days and 28 days. Eight animals of experimental study period from each group were dissected in both durations. After every duration serum, Broncho alveolar lavage fluid and lung tissue were collected from each animal to evaluate physiological, immunological, molecular genetics, histological and histopatholgical alterations.

All results were compared with the corresponding normal control group then after, bleomycin treated groups were further compared with bleomycin group. The present findings were then discussed in view of the relevant literature available in similar fields of study.

The present study elucidated a significant decrease in body weight inC57BL/6 after intratracheal BLM instillation for 28 days. This was accompanied with a significant elevation in lung weight, lung index, serum MDA and LDH either after 14 or 28 days of study period. In addition, there was an elevation in lung tissue collagen profile, inflammatory mediators. Moreover, Caspase-3 and Muc5ac gene expressions showed a significant increase while the gene expression of BCL2 was declined. Furthermore, a significant increase of total leukocytes thus increased inflammatory cell counts in BALF of neutrophils, lymphocytes and macrophages were also established after both 14 and 28 days of bleomycin instillation. Besides, recognized histopathlogical alterations in mice lung sections following were seen.

Nano chitosan nanoparticles as a noval nano targeted treatment for bleomycin-induced pulmonary fibrosis in male C57BL/6 mice model were analyzed by SEM, TEM, EDXA and FTIR. These analyses were carried out for Nanochitosan before and after loading with dexamethasone. Also SEM and TEM confirmed that dexamethasone was adsorbed on the surface and the absorbed inside Nano-chitosan. While EDX analysis confirmed the elements content in each component, DEX, NCH and DEX-NCH particles. Furthermore, FTIR proved all functional groups presented in DEX, NCH particles and DEX-NCH particles. The treatment with DEX alone or DEX loaded on NCH particles or under DEX and NanoCURC produced a significant decline in lung weight, lung indices, serum LDH and MDA. This was also accompanied by a decrease in the inflammatory mediators including TNFα, TGF β, INFγ, NFkB and lung tissue inflammatory marker MMP2. In addition, microscopical investigation for total leucocytic count and differential cell counts of macrophages, lymphocytes and neutrophils in BALF revealed likewise a