Formulation of Stimuli-Sensitive Thiolated Hyaluronic Acid Based Nanofibers: Synthesis, Characterization, Preclinical Safety and In Vitro anti-HIV Activity

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Purpose: It was hypothesized that a bio-responsive and mucoadhesive hyaluronic acid-nanofibers (HA-SH-NFs) can be topically safe nanocarriers for the effective prevention of HIV virus transmission.

Methods: A novel sulphydryl (-SH) group modified thiolated-HA was synthesized and characterized (FT-IR & NMR analyses) to fabricate the TFV-loaded-HA-SH-NFs using electrospinning method. The surface morphology and size distribution of NFs was determined using scanning electron microscopy. The in vitro mucoadhesion of NFs was analyzed by ellipsometer and mucin-interaction assays. The in-vitro cytotoxicity of NFs was analyzed on cervicovaginal (CV) cells and Lactobacillus bacteria. In-vivo safety of NFs upon once-daily vaginal administration up to 7days was assessed by histological analysis in female C57/BL6 mice. The inflammatory cells (CD45) infiltration in genital tract up to 7days of treatment of NFs was determined by immunohistochemistry assay. The cytokine (IL-1α, IL-1β), IL-6, IP-10, IL-7, MKC, TNF-α levels (pg/mL) in CV lavage and tissues were analyzed. The in-vitro anti-HIV activity of NFs was analyzed using pseudo typed HIV virus-particles (mean diameter ~128nm, and titer ~3.07x10¹⁰ particles/mL) determined by nanoparticle tracking analysis. The anti-HIV activity was analyzed using a luciferase assay using different concentrations of free TFV and TFV loaded NFs.

Results: Thiolated HA-SH were synthesized and SEM images showed the formation of HA-SH-NFs in the mean diameter of ~75 nm. A higher mucoadhesion of NFs was observed compared to the native-HA based on an increase in the size (~4fold), thickness (~3fold) and adsorbed mucin amount (~2fold) after 3h incubation with mucin. A semen hyaluronidase enzyme triggered drug release (~87%w/w) from NFs occurred after 1h. The NFs were non-cytotoxic to CV cells and L. crispatus bacteria. Histological data showed no damage on the mice genital tract (vagina, cervix, uterus, ovary, rectum), and spleen, lung, liver, kidney, heart, brain tissues on exposure to NFs. Following 24h of exposure, no significant CD45 cell-infiltration and the changes in cytokines levels were observed compared to control mice. Anti-HIV activity data suggested that the TFV-loaded-NFs were able to inhibit the virus replication and the structural-integrity of TFV was unaffected by the composite geometry of NFs.

Conclusions: The data represented here highlight the potential of bio-responsive HA-SH-NF templates for the safety and vaginal delivery of anti-HIV/AIDS microbicides under the influence of seminal hyaluronidase enzyme. 

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References: