Hyaluronan Based Pendant-Chain System for Delivery of Chemotherapeutic Drugs

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Statement of Purpose: Targeted drug therapy has been the focus of research in the past two decades with the goal of reducing systemic toxicity and improving the localized therapeutic efficacy of drugs. Because of its high socio-economic impact, cancer is one of the diseases being investigated for targeted drug therapy. Hyaluronan, a hydrophilic biopolymer, has been investigated as a pendant-chain delivery system since it offers several advantages including high water solubility, receptor mediated targeting of CD44 and RHAMM, receptors for hyaluronan that are over-expressed by many carcinomas (Savani RC. J Biol Chem. 2001; 276: 36770-36778), and the availability of functional groups for drug conjugation. Chemotherapeutic agents 3-(5-methoxy, 2 methyl-1H-indol-3-yl)-1-(4-pyridinyl)-2-propen-1-one (MOMIPP) and resveratrol have been conjugated to hyaluronan with the help of simple carbodiimide interaction. MOMIPP and resveratrol has been conjugated hyaluronan and HA-TAMRA to verify receptor-ligand interaction. NMR and TLC results suggest successful conjugation of MOMIPP and resveratrol to hyaluronan, and the pendant-chain delivery systems has high water solubility.

Methods: Cancer cells were perfused with fluorescently-tagged hyaluronan (HA-TAMRA) in a parallel plate flow chamber (Glycotech, MD) and imaged with a fluorescent and a confocal microscope to determine the attachment and uptake of hyaluronan under physiological flow conditions. A competitive attachment experiment was performed similarly with a mixture of un-labeled hyaluronan and HA-TAMRA to verify receptor-ligand interaction. MOMIPP and resveratrol has been conjugated to hyaluronan with the help of simple carbodiimide chemistry, and the final conjugates were characterized with nuclear magnetic resonance (NMR) and thin layer chromatography (TLC). In vitro targeting potential was determined by an alamarBlue® cell viability assay (Invitrogen, CA) after a 72 hours of drug incubation. A cell relapse study, designed to study the regrowth of cells, was performed with a LIVE/DEAD® cell viability assay (Invitrogen, CA) by incubating cells with drug-free media after the initial 72 hours of drug incubation. An orthotopic breast tumor model using athymic nude mice was tested to determine the in vivo efficacy of the conjugates. Tumor bearing mice were injected with HA-TAMRA via tail vein, and the fluorescence intensity from the harvested organs was measured to determine the targeting potential of hyaluronan in vivo.

Results: Breast cancer MB231, MB157, and MCF7 cells show a significantly higher attachment (p < 0.05) of HA-TAMRA under physiological flow compared to the non-cancerous controls. Results from confocal microscopy confirm the uptake of hyaluronan upon attachment with their target receptors. Additionally, aggressive breast cancer MB231 do not show any reduction in attachment of HA-TAMRA under physiological flow conditions in competition with 2.4 mg/ml un-labeled hyaluronan, while non-aggressive MCF7 cells show a significant reduction (p < 0.05) in attachment under similar conditions. NMR and TLC results suggest successful conjugation of MOMIPP and resveratrol to hyaluronan, and the pendant-chain delivery systems has high water solubility. In vitro results from the alamarBlue assay suggest 12 to 25 fold increases in toxicity of the HA-resveratrol compared to the parent drug for MB157 and MB231, respectively. HA-MOMIPP shows 2 to 8 fold increases in toxicity for MB231 and MCF7, respectively. As expected, the hyaluronan based pendant-chain delivery systems eliminate the use of organic solvents that are required to solubilize these drugs. Additionally, minimal toxicity is observed with non-cancerous controls. Another advantage of conjugating drugs to hyaluronan is evident from the cell relapse study, where cells incubated with the conjugates do not show signs of regrowth, while cells incubated with drugs regrow upon incubation with fresh media. Finally, breast carcinomas harvested from animals show 35-fold higher accumulation of hyaluronan compared to the kidneys and livers. This result re-enforces the targeting advantages of hyaluronan based pendant-chain delivery system and encourages their further development.

Conclusions: The conjugation of both MOMIPP and resveratrol to hyaluronan increases efficacy of both drugs. This result is due to changing the drug’s solubility from organic to aqueous solutions. As a result the drug’s bioavailability and the ability to target receptors are significantly enhanced. These characteristics are translated into in vivo studies where the breast carcinoma accumulates the hyaluronan based pendant-chain delivery system. Thus, hyaluronan is a versatile platform for targeted cancer therapy for a wide variety of chemotherapeutic agents.