Development of food derivative therapeutic ellagic acid-chitosan based delivery systems for local chemotherapy

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Statement of Purpose: Recently, many researches have focused on designing polymer devices that deliver chemotherapeutics into surgical resection cavity of the tumor tissue. Addition of a polymer to a cancer drug physically or chemically creates much higher local drug concentrations and makes it possible to place the drug next to targeted tissue. The objective of this study was to develop chitosan based delivery systems to locally deliver ellagic acid to effectively treat cancers. Ellagic acid is currently used as a dietary supplement to reduce risk of cancer due to its anti-carcinogenic, -mutagenic, -microbial, and -angiogenic properties. Chitosan can be readily formed in film-type and gel-type and is promising in the development of locally controlled-release systems. Our study showed the feasibility of chitosan-ellagic acid (Ch-EA) composite materials for local chemotherapy. These materials inhibited proliferation of human WM115 melanoma, human U87 glioblastoma, and rat C6 glioma cells *in vitro* and significantly inhibited rat C6 glioma growth *in vivo*. Currently, we have focused on the transformation of chitosan based formulation into thermally sensitive gel forming solution and delivery of ellagic acid by simple injection into the cavity of the body after surgical removal of tumor tissue, thereby improving both the safety and efficacy of cancer treatment.

Methods: Chitosan (190- 310 KDa, 85% DDA) based composite films were prepared by solution casting 1%(w/v) chitosan with different concentration of ellagic acid while chitosan based thermo-sensitive formulations were prepared on the basis of the combination of chitosan and beta-glycerophosphate disodium salt (β-GP). The chitosan-ellagic acid composite materials were characterized using FTIR, XRD, SEM, and contact angle measurement. Release rate of ellagic acid from the composite materials was examined and enzymatic degradation rate was determined by analyzing the increased free amino groups in the incubation medium. Cell viability was tested through direct and indirect cell culture on the composite films by MTS assay. To analyze the anti-proliferative mechanism of the films on cancer cells after treatment, apoptosis assay, western blot, and anti-angiogenesis assay were performed. In the animal study, GFP tagged rat C6 glioma cells were implanted subcutaneously at the right flank region of nude mice and allowed to grow until tumor size reaches 2cm in a diameter. Treatment was initiated by implanting either a chitosan film (a carrier) or Ch-EA20 film subcutaneously on the fifth day after tumor injection. Tumor growth was evaluated by measuring tumor volume using a caliper, an ultrasound machine, and an optical imaging system.

Results/Discussion: Our study demonstrated that with the increasing concentration of the ellagic acid, chitosan-ellagic acid composite films (Ch-EA) formed new chemical bonds, became rougher and more hydrophilic, and possessed increased crystallinity of ellagic acid. Release profiles of ellagic acid from films showed a sustained release of ellagic acid over the experimental time periods, and lysozyme could promote the release profile of ellagic acid. Enzymatic degradation rate of films was dependent on the weight percentage of chitosan and the concentration of ellagic acid, which affected the structure of the polymer networks chemically and physically. The effect of films on viability of cells (human WM115 melanoma, human U87 glioblastoma, and rat C6 glioma cells) was dependent on cell types and concentrations of ellagic acid. Films could induce the accumulation of the tumor suppressor protein p53 and increase caspase-3 activation, which preceded an induction of apoptosis. The animal study demonstrated that Ch-EA20 group significantly inhibited tumor growth compared with the untreated group and chitosan control group. These results allowed us to further the chitosan based delivery system for the use of ellagic acid locally to treat cancer cells.

Conclusions: Chitosan-ellagic acid composite films could deliver ellagic acid locally with sustained slow release rate and presence of lysozyme could promote the release profile of ellagic acid from films. These materials could induce apoptotic cell death in an ellagic acid concentration dependent manner and significantly inhibited tumor growth *in vivo.*

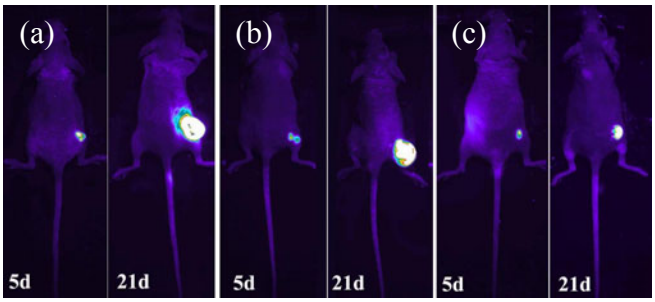

Figure 1. FRI images of nude mice with GFP (green fluorescent protein) tagged C6 rat glioma in each right flank on 5 and 21 days after tumor inoculation: (a) Untreated, tumor control; (b) Chitosan, carrier control; (c) Ch-EA20, experimental group. Tumor volumes were measured by an optical imaging measurement. Caliper and ultrasound measurements (not shown) have similar results.

References:

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