Self-Assembled Micellar Complex Comprised of Green Tea Catechin Derivatives for Protein Drug Delivery

Susi Tan, Joo Eun Chung, Shu Jun Gao, Motoichi Kurisawa and Jackie Y. Ying

Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos, Singapore 138669

Statement of Purpose: The drug-to-carrier ratio has been a considerable issue in the design of a drug carrier because the use of high quantities of carrier can lead to problems associated with carrier toxicity, metabolism and elimination[1]. However, if the carrier itself displays therapeutic effects, the drug-to-carrier ratio would no longer be a concern, and the delivery system should attain greater therapeutic effects from both the drug and the carrier. (-)-Epigallocatechin-3-O-gallate (EGCG), a major ingredient of green tea, is recognized for its various potential therapeutic effects, including anticancer effects. Herein we introduce the core-shell micellar nanocomplexes (MNCs) spontaneously constructed by the self-assembly of EGCG derivatives and therapeutic protein. This system is the first to utilize EGCG as a carrier for biological molecules aiming at the synergistic therapeutic effects associated with the carrier itself.

Methods: OEGCG and PEG-EGCG were synthesized by the Baeyer reaction between an aldehyde group and a nucleophilic A ring of EGCG in acidic condition. The self-assembly of micelles was achieved with the sequential addition of OEGCG and PEG-EGCG to the protein solution. The size and polydispersity of complexes were evaluated by dynamic light scattering (DLS). The activities of various proteins during the micelles' complexation and dissociation process were examined. The anticancer efficacy of the MNCs was assessed by cell proliferation study *in vitro* and tumor regression study *in vivo*.

Results: We have successfully formulated uniform, spherical MNCs (ca. 90 nm) by sequential addition of OEGCG and PEG-EGCG to the protein solution; the PEG-EGCG acted as the shell for the OEGCG/protein complexes (**Fig 1**). Ideally, the MNCs need to stably protect the proteins from degradation during delivery, while exerting their own therapeutic activities at the sites of delivery. We observed that the activities of various proteins were restrained by the complexation with OEGCG. However, the activities were fully restored when the nanocomplexes were dissociated.

The MNCs were loaded with Herceptin (trastuzumab), a monoclonal antibody that induces regression of HER2-overexpressing metastatic breast cancer tumors. The anticancer effect of the MNCs loaded with Herceptin was explored *in vitro* and *in vivo*. The cancer cell growth inhibitory effect was observed on BT-474 after treatment with free Herceptin, Herceptin-loaded micellar nanocomplex (Herceptin-MNC), bovine serum albumin-loaded micellar nanocomplex (BSA-MNC) (i.e. drug-free carrier), and an equivalent amount of each carrier component (OEGCG and PEG-EGCG). BSA-MNC showed cancer cell growth inhibitory effect due to the anticancer effect of OEGCG and PEG-EGCG; whereas BSA showed no effect. Notably, Herceptin-MNC showed a higher inhibitory effect than free Herceptin via synergism of the inhibitory effects of the carrier and Herceptin.

The anticancer effect of Herceptin-MNC was also investigated *in vivo* using a BT-474-xenografted nude mouse model. The tumor treated with phosphate buffered saline (PBS) (vehicle control) progressed rapidly for 34 days. In contrast, Herceptin-MNC efficiently retarded tumor growth; in fact, complete tumor regression was observed in some mice. Herceptin-MNC showed a significantly higher anticancer effect than free Herceptin. During the period of treatment, the mice did not show loss in body weight and signs of toxicity The results demonstrated the significant benefit of utilizing Herceptin-MNC in the BT-474 model, which could be attributed to the effects of combining Herceptin and the green tea-based MNC.

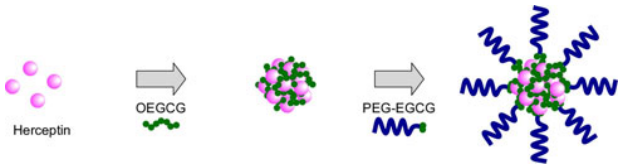

Figure 1. Schematic diagram of self-assembled micellar nanocomplexes loaded with proteins.

Conclusions: The green tea-sourced MNCs loaded with an anticancer protein successfully demonstrated a much greater anticancer effect than the free protein *in vitro* and *in vivo*. This MNC offers the possibility of improved delivery of various therapeutic molecules that EGCG can bind to. It provides an additive therapeutic effect associated with the versatile green tea-sourced carrier.

References:

1. Allen, T.M., et al., Science 2003; 303:1818-1822.
2. Matsumura, Y., et al., Cancer Res. 1986; 46:6387-6392.
3. Duncan, R. Nature Rev. Cancer 2006; 6:688-701.