151c Cytotoxic Activity, Transport, and Drug Release Mechanisms of Dendrimer- Methotrexate Conjugates on Sensitive and Resistant Cancer Cell Lines

Sezen Gurdag, Rangaramanujam M. Kannan, Sarah Staples, and Larry Matherly

Dendrimers are tree-like, globular-shaped polymers with densely packed end-functional groups that can be used to attach bioactive molecules such as drugs, targeting ligands and imaging agents. Cancer is one of the applications of these unique materials. Since significant portion of administered dose of anticancer drugs is lost in the circulation due to impaired uptake by the cells especially in the case of drug resistance cells or P-gp related mechanisms, the actual concentration of the drug inside the cells is much less than what is present extracellularly. Hence, to accomplish highly effective cancer treatment it is crucial to increase intracellular amount of the drug. In this research, we synthesized dendrimer-anticancer drug conjugates for activity evaluation on two drug sensitive and two drug resistant cell lines.

Two conjugates of methotrexate (MTX, an antimetabolite drug) with generation 3 and 2.5 polyamidoamine (PAMAM) dendrimers were prepared using DCC as coupling agent. Conjugate A was prepared by DCC coupling the -NH2 group of MTX to a PAMAM-G2.5-COOH dendrimer, while Conjugate B was prepared by coupling the -COOH group of MTX to a PAMAM-G3-NH2 dendrimer. Both involved the formation of an amide bond between the drug and the dendrimer. It has been shown that less than 5% of MTX can go inside the cells even after 4 hrs. We aimed to increase the cellular uptake of the drug via conjugation by changing the transportation route of the drug from the receptor-mediated (RFC) transportation to endocytosis, which will be dominated by the characteristics of the dendritic carrier. The conjugates were tested on both MTX-sensitive and MTX-resistant human acute lymphoblastoid leukemia and Chinese hamster ovary cells. The activity of the conjugates was investigated on both MTX-sensitive and resistant human acute lymphoblastoid leukemia (CCRF-CEM) and Chinese hamster ovary (CHO) cell lines. To assess the antifolate activity, cells were treated with free MTX or conjugated MTX along with nucleosides as protection agents (adenosine and thymidine).

Based on IC50 values, PAMAM-G2.5COOH-MTX conjugate (Conjugate A) has been shown to have similar level of cytotoxic activity in sensitive PC43-10 cells while the activity was enhanced by the factor of 3 against CCRF-CEM cells in comparison to free MTX. On the other hand, the conjugate demonstrated 8-24 times better response in resistant CEM/MTX and RII cell lines, respectively. This is a very surprising result in a non-targeted nanodevice, since in previous literature where targeting ligands were not used, the conjugates mostly show LOWER cytotoxicity. As opposed to high activity obtained with Conjugate A, the conjugate of MTX prepared with PAMAM-G3-NH2, Conjugate B, was mostly inactive against the cell lines. Even though the conjugates have amide linkages, the difference in two conjugates can be explained by different MTX release characteristics from the conjugates. We hypothesize that the anionic Conjugate A is most likely releasing the drug more efficiently compared to the cationic Conjugate B. This is strongly suggested by the higher activity of this conjugate observed in almost all the cell lines investigated. Anionic nature of PAMAM-G2.5-COOH may be making the dendrimer stay in the lysosomes for long enough to release the drug while cationic PAMAM-G3-NH2 dendrimer may be pumped out as a result of high concentration of H+ ions present in the lysosomal compartments. This will be investigated by studying comparative localization of the dendrimers particularly in the lysosomes by confocal microscopic visualization technique where lysosomes will be tracked by specific markers with high selectivity for acidic organelles. This work is in progress. For further characterization of the conjugates, the cells were co-incubated with the conjugated drug in the presence of adenosine and thymidine nucleosides used as rescue agents. Approximately 70% of the cells were viable when supplied with the nucleosides as a result of bypassing the pathways where the activity of DHFR is needed. Employing the salvage pathways was the indication of a similar mode of action displayed by the conjugates to that of the free drug. This is an indication that over the 96 hours, the drug is released intra-cellularly. Our preliminary flow cytometric analysis for the cellular entry of FITC-

attached PAMAMG2.5COOH dendrimer showed that the dendrimer is internalized by the RII cells as fast as 15 minutes. In summary, it has been shown that conjugation of MTX through its -NH2 group to -COOH-terminated dendrimer resulted in a conjugate with higher cytotoxic activity than free MTX. However, conjugation with PAMAM-G3-NH2 dendrimer decreased the cytotoxicity of the parent drug.

Current work focuses on the evaluation of the conjugates in animal models of B-cell lymphoma. Conjugates of dendrimers with folic acid targeting ligands and the drug are also being synthsized for in vitro and in vivo evaluation.