J. Pharm. Pharmacol. 1998, 50 (Supplement): 102

Preliminary characterization of amino acid based polymeric vesicles for gene delivery

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There is an expanding array of non-viral delivery systems being developed and studied for the delivery of genetic material into cells. However, many of these systems have various disadvantages with only a few having substantial success in vivo, Schofield & Caskey (1995). Therefore, there is an ever-present need for the discovery of new delivery systems, which aim to overcome these existing problems and ultimately encompass all the required parameters for a highly effective gene delivery system. Cationic polymers have been tested previously as gene delivery agents, Chowdhury et al (1993), and our studies are concerned with the evaluation of cationic polymer based vesicles for gene delivery. Poly-L-lysine and poly-L-ornithine homopolymers have been modified by the covalent attachment of palmitoyl and polyethylene glycol (PEG, MW~5,000) groups (initial ratio of amino acid to palmitoyl and PEG groups = 4:1 and 20:1, respectively) to yield the amphiphilic polymers PLP (in the case of poly-L-lysine) and POP (in the case of poly-L-ornithine).

Characterisation of the polymer systems was carried out by NMR and FT-IR spectroscopy. These cationic polymers were found to assemble into vesicles in the presence of cholesterol in a similar manner to palmitoyl glycol chitosan, Uchegbu et al (1998). Both POP and PLP vesicles were prepared by sonication with cholesterol in phosphate buffered saline (PBS, pH=4). The vesicles were visualised by freeze fracture and negative staining transmission electron microscopy. In vitro studies were carried out on the A549 (lung) human carcinoma cell line. Biocompatibility of these systems was investigated using the MTT assay. DNA condensation by these vesicles was probed using ethidium bromide and transfection experiments carried out with the endotoxin free pCMV-sport-β-gal plasmid.

These systems are able to condense DNA at a ratio of 10:1 or above and transfer DNA into mammalian cells (Figure 1). Long-term stability was also observed with these polymeric vesicles as they could be visualised by freeze-fracture electron microscopy after storage for 9 months at refrigeration temperatures.

These results are promising and future work is aimed at developing these agents for in vivo gene delivery.

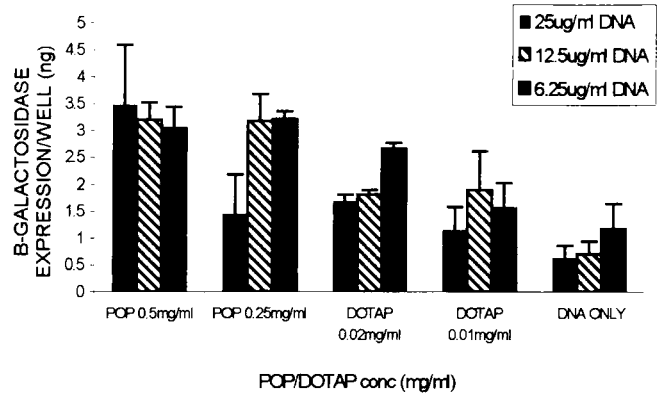

FIGURE 1 – B-galactosidase protein levels after transfection of A549 cells with POP: chol vesicles and DOTAP vesicles complexed to β-gal plasmids.

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