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Novel cholesterol based siRNA lipoplexes with and without PEG-modification: Characterization and *in vitro* cytotoxicity studies

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Cationic liposomes have potential in carrying small interfering RNA (siRNA) molecules as gene medicines. However, unfavorable liposome-serum interactions often limit their efficacy. In order to address this concern, liposome-stabilizing agents, cholesterol (Chol) and polyethylene glycol (PEG) were incorporated in the design of new liposome-siRNA systems. The helper lipid, Chol, was combined in equimolar quantities with the cytofectin, N,N-dimethylaminopropylamidosuccinylc holesterylformylhydrazide (MS09), to give unilamellar vesicles. For PEG-modification, distearoylphosphatidylethanolamine poly(ethylene glycol) 2000 was added at 2 mol %. Electrostatic association of liposomes with siRNA was followed in band shift and fluorescence quenching assays. Liposome-siRNA complexes (lipoplexes) were observed as globular aggregates by cryo-transmission electron microscopy. Characterization of lipoplexes by Zeta-potential Nanoparticle Tracking Analysis (Z-NTA) showed that lipoplex size and zeta potential were dependent on both liposome composition and the MS09: siRNA (w/w) mixing ratio. siRNA within lipoplexes resisted serum-induced damage at MS09:siRNA (w/w) ratios of 12:1-32:1. The effects of lipoplexes on cell growth were evaluated with a non-targeting siRNA sequence in transformed and non-transformed human cell lines. MTT and alamarBlue® assays showed that MCF-7 and HEK293 cells retained at least 78% viability at final siRNA and lipid concentrations of 57 nM and 29-60 μM, respectively. In general, cell survival profiles of MS09/Chol and MS09/Chol/PEG liposomes compared favorably with that of Lipofectamine™ 3000 and control formulations which contained the conventional helper lipid, dioleoylphosphatidylethanolamine (DOPE). At present, the siRNA delivery capability of liposomes is under assessment and the most promising formulations will be applied to the delivery of oncogene-specific siRNA in gene silencing experiments.

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Structural and functional study of a novel cathelicidin antimicrobial peptide BuMAP-28 and its analogue (BuMAP-28)18

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Cathelicidin antimicrobial peptides BuMAP-28 and (BuMAP-28)18 were synthesized and used for the present study. Amino acid sequences of the antimicrobial domain were deduced from the gene sequence of myeloid antimicrobial peptide of buffalo. Peptides are highly cationic, amphipathic showed a net charge of +11 for both BuMAP-28 and its analogue; a predicted hydrophobic ratio of 39% for BuMAP-28 and 33% for (BuMAP-28)18. Ramachandran plot analysis indicating a high structural stability for the peptides. *In silico* structural analysis of the peptides revealed a helix-turn helix which is later confirmed by CD analysis. Biological activity testing of the peptides revealed that the peptide has got a broad spectrum antimicrobial and anti-cancerous activity. Peptides showed wide spectrum of activity against Gram-positive, Gram-negative bacteria, fungi, spirochetes and virus. Peptides are active against even methicillin resistant *S. aureus* with lower MIC values. *In vitro* antibacterial and antifungal activities were later confirmed by morphological testing and SEM. Peptides are also proved to be effective against HeLa cell lines. Cytotoxic studies revealed that the truncation of BuMAP-28 could reduce the hemolytic activity when compared to the parent peptide. Murine models injected with Duck *Pasteurella* (DP1), when treated with the peptides protected 100% of the animals at 12.5 μM doses. Studies revealed that the truncation of the peptide could reduce the hemolytic activity without a considerable change in antimicrobial activity.

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