in 95% loss of covalently bound PBD. Interestingly, acidic conditions (*e.g.,* TFA) were not required as in the experiments conducted by Hurley and co-workers with anthramycin. Within a 10 minute cooling period at 0ºC, GWL-78 was observed to re-attach to the DNA. Furthermore, the rate of re-attachment was found to depend upon the sequence of the oligonucleotide. For example, with AT-rich oligonucleotides, 85% of the adduct had re-formed within 10 minutes, while only 30% had re-formed with GC rich sequences. Although PBDs generally prefer to bind to GC rich sequences of DNA, the latter result most likely reflects the relatively high affinity of GWL-78 for AT-rich DNA sequences due to the *bis*-(*N*-methylpyrrole) component it contains. Further investigations showed that although the rate of re-formation of the PBD-DNA adduct depended on DNA sequence, initial cleavage of the adducts by heat did not. These observations add to knowledge of PBD chemistry and mechanism of action, and may help in the design of future PBD-based gene-targeting agents and anticancer and antibacterial agents.

529 POSTER

Endocytosis and intracellular trafficking of cholesterol based cationic liposome for gene delivery

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Background: For the effective gene therapy or transfection, it is important not only to prepare the genetic materials such as plasmid DNA and siRNA but also to deliver these molecules to target cells. Using cationic liposome to mediate gene delivery is widely used because of the advantage of the safety and simplicity of use over viral gene therapy. Recently, we developed and published the cholesterol derived cationic lipids with ether linkers, and these showed more efficient gene delivery compared to commercial lipids such as DOTAP and lipofectamine. Therefore, we investigated the endocytosis and trafficking of this cholesterol based cationic lipids.
Material and Methods: We compared the endocytosis and trafficking of this cholesterol based cationic lipids with DOTAP as non-cholesterol based lipid in COS7 cells. We investigated the effects of several inhibitors of particular routes on the uptake of lipoplexs by flow cytometry and subsequent gene expression by luciferase expression assay and GFP observation using fluorescence microscopy. The involvement of late endosome and lysosome were verified by the colocalization of the fluorescence labeled plasmid and the antibody to EEA1 and LAMP1 in both lipids.
Results: Lipoplex internalization of both lipids was inhibited by clathrin pathway inhibitor chlorpromazine, but unaffected by caveolar pathway inhibitors such as filipin and genistein. The observation using confocal laser microscope showed the both of fluorescently labeled lipoplexes were co-localized with transferin known as clathrin pathway marker. The involvement of late endosome and lysosome were verified by the colocalization of the fluorescence lableled plasmid and the antibody to EEA1 and LAMP1 in both lipids. However, the decreased efficiency of transfection by lysosomal inhibition with chloroquine called the needs for further study. Subsequently, we found that the transfection efficiency of DOTAP was more inhibited by the depletion of membrane cholesterol with methyl-beta-cyclodextrin compared to newly synthesized cholesterol based lipids. Furthermore, the replenishment of cholesterol and our cholesterol based lipids restored the transfection efficiency of DOTAP.
Conclusions: In conclusion, we found that our cholesterol-based cationic lipids were internalized by clathrin-mediated pathway and the substitutive effects of cholesterols might be one cause of the superiority of this delivery vehicle.

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Activity of the TGF-beta 2 specific antisense oligodeoxynucleotide trabedersen in an orthotopic xenograft mouse model of metastatic pancreatic cancer

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Background: Transforming growth factor-beta (TGF-beta) regulates crucial cancer mechanisms such as cell proliferation, metastasis, angiogenesis, and immunosuppression. The isoform TGF-beta 2 plays a particular role in some cancers e.g. pancreatic cancer. Our novel therapeutic approach to treat cancers overexpressing TGF-beta 2 is based on the specific inhibition of its synthesis by the antisense oligodeoxynucleotide trabedersen (AP 12009). In the present study, the antitumor activity of trabedersen was investigated in an orthotopic xenograft mouse model of metastatic pancreatic cancer.
Material and Methods: BALB/C$^{nu/nu}$ mice were injected with 1×10^6 cells of the human TGF-beta 2 expressing cell line L3.6pl into the pancreatic tail. Intraperitoneal treatment with trabedersen or vehicle (controls) started 2 days after tumor implantation with an initial loading dose of 50 mg/kg and subsequent doses of 16 mg/kg thrice weekly. On day 29, mice received BrdU by intraperitoneal injection and were sacrificed 2 h thereafter. The incidence of liver and lymph node metastases as well as size and weight of the pancreatic tumors were determined. Tumor sections were stained with anti-BrdU antibody to determine tumor cell proliferation and with anti CD31/PECAM-1 antibody to determine vascularization.
Results: Tumor weight in trabedersen-treated mice was significantly reduced compared to control mice (mean tumor weight: 0.7 g vs 1.4 g, $p = 0.0084$). Concordantly tumor cell proliferation was significantly suppressed ($p = 0.028$). Lymph node metastases were detected in most (7 of 9) control mice but only in 2 of 10 trabedersen-treated mice ($p = 0.023$). Liver metastases were present in 5 of 9 control mice and in 4 of 10 trabedersen-treated mice (ns). Vessel area in tumor slices as readout for tumor angiogenesis was significantly reduced in trabedersen-treated animals to about a third of the vessel area of control animals ($p = 0.0001$).
Conclusions: In an orthotopic xenograft mouse model of metastatic pancreatic cancer trabedersen demonstrated potent antitumor activity. Key tumor promoting mechanisms such as tumor cell proliferation, metastasis and tumor angiogenesis were efficiently suppressed by trabedersen. This is in line with promising results from clinical studies of trabedersen in patients with advanced pancreatic cancer (phase I/II) or high-grade glioma (randomized, controlled phase IIb completed, phase III ongoing).

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Host cell specific responses to a novel rVSV oncolytic vector rNCP12.1

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Background: Vesicular stomatitis virus (VSV) is a -ssRNA virus known to be inherently oncolytic due to defects in tumor interferon (IFN) response pathways. This study addresses host specific responses and how they contribute to the oncolytic potential of rNCP12.1, a novel recombinant VSV shown to decrease intracranial tumors in an immunocompetent rat glioma model by 67%.
Methods: To determine tumor specificity of rNCP12.1, we performed MTS cell viability assays following viral infection from 6 hours (hpi)-1 week post-infection (wpi) in rat glioma cells (F98) and in primary rat astrocytes (PRAs). Supernatants were used to determine differences in replication between normal and tumor cells. To examine the differences in the ability of glioma and normal cells to elicit an antiviral IFN response, we performed IFN protection assays. IFN bioassays were used to test the host specific production of IFN in response to activation of both TLR7 and RIG-I pathways by transfected polyI:C (tpI:C) and viral infection, respectively.
Results: rNCP12.1 maintained wtVSV level cytotoxicity in F98 glioma, with majority of cell death seen by 48 hpi. rNCP12.1 displayed minimal cytotoxicity in PRAs throughout the study. Growth curve data supported these trends, showing highly permissive replication of both viruses in F98 and significantly restricted replication of rNCP12.1 in PRAs. Following IFN exposure, C6 glioma were partially protected from infection but required 4 times more IFN than PRAs; F98s were not protected with any amount of IFN. Following infection with rNCP12.1, rat fibroblasts (FR) produced high levels of IFN. C6s produced extremely low amounts of IFN while no IFN was detected from F98s. No IFN production was detected in wtVSV-infected cells of any type. tpI:C production of IFN paralleled results from IFN bioassays of virally infected cells but at 3−20 fold lower amounts for each cell type.
Conclusions: Though there are obvious host specific differences in IFN responses following rNCP12.1 infection among normal and tumor cells and even between similar glioma cell types, the overall inability of glioma cells to elicit a sufficient antiviral IFN response contributes to the oncolytic capacity of this vector. Based on these differences, we believe rNCP12.1 is an attractive candidate for treatment of glioma and further preclinical studies are being conducted.