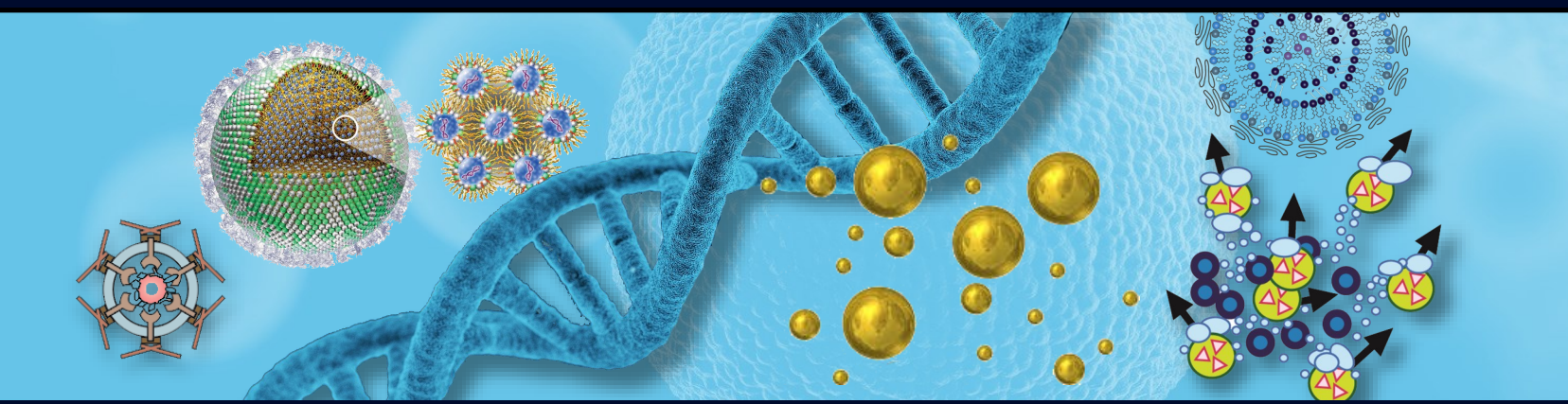
ABSTRACTS

Adjudicated Poster Competition Abstract Compendium



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Abstract #1

Optimizing siRNA delivery to breast cancer with novel ionizable lipid

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Background

The development of ionizable lipid (IL) was imperative to enhance the effective formulation of small interfering RNA (siRNA) to inhibit P2X7 receptors (P2X7R), a key player in tumor proliferation, apoptosis, and metastasis. The synthesis and utilization of IL facilitates the cellular uptake of lipid nanoparticles (LNP), thereby improving the efficient delivery of siRNA-LNPs to downregulate the overexpression of P2X7R.

Methods

To evaluate the impact of P2X7 knockdown on breast cancer (BC) cell behavior, we designed a novel synthesized ionizable lipid (SIL) to enable the effective transfection of siRNA-LNP targeting P2X7 receptors (siP2X7) in mouse 4T-1 cells. The SIL was synthesized and characterized. Following the assessment of the LNP stability (residual lipids) through HPLC-ELSD and the determination of toxicity for SIL and siP2X7-LNP using the MTT assay, the cellular uptake of siP2X7-LNP was visualized using confocal microscopy. After LNP characterization, the determination of siRNA encapsulation, dosage, time incubation, migration inhibition, and apoptosis induction with scratch assay and flow cytometry were analyzed, respectively. Finally, the total expressed protein of P2X7R was measured using western blotting.

Results

The resulting formulated LNP with SIL exhibited favorable properties, including a Z-average of 126.8 nm, Zeta-potential of -12.33, PDI of 0.16, and encapsulation efficiency of 85.35%, after determining the optimal siP2X7 dose (45 pmol). Integrating the innovative branched SIL into the formulation of siP2X7-LNP led to significant migration inhibition (0.2 μm scratch area and 50% healing closure) and apoptosis induction (late apoptosis 58% and necrosis 36%) at 48 h in 4T-1 cells due to enhanced cellular uptake. The results demonstrated the pivotal role of the SIL in efficiently delivering siRNA against murine triple-negative breast cancer cells (TNBC) using LNP formulation, resulting in notable efficacy.

Conclusions

The present research examined the incorporation of SIL into prepared LNP with efficient siRNA delivery to silence P2X7R in 4T-1 breast cancer cells. The solvent-free method in synthesizing symmetric and branched SIL demonstrated improved effectiveness and biocompatibility for IL in LNP formulation. Consequently, the developed siP2X7-LNP(SIL) formulation against P2X7R successfully promoted metastasis suppression and induced apoptosis in 4T-1 cells, and western blotting analysis confirmed the overall protein expression level of P2X7R after treatment with siP2X7-LNP.

Abstract #2

Development of a novel polymer for boosting lipid nanoparticle delivery of nucleic acids

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Background

Lipid nanoparticles (LNPs) have emerged as promising carriers for delivering nucleic acids. However, less than 2% of the nucleic acid payload is mostly being released in the cytosol by the LNP-mediated delivery.[1] To address this major barrier, we have synthesized a novel polymer (LR) that can destabilize lysosomes and be incorporated within LNPs.

Methods

In this study, we optimized LNP formulations by incorporating varying amounts of the polymer and assessed these formulations through dynamic light scattering to determine particle size and polydispersity, zeta potential measurement by electrophoresis, and mRNA encapsulation efficiency via the RiboGreen assay. We also assessed the *in vivo* bioluminescence of mRNA transfection using the polymer modified LNPs.

Results

The resulting polymer-incorporated LNPs exhibited physicochemical properties comparable to those of standard LNPs with sizes ranging within 65-85 nm, with a polydispersity index (PDI) less than 0.2. The encapsulation efficiency of the polymer-incorporated LNPs was 90%. The *in vitro* results of eGFP loaded polymer-incorporated LNPs displayed increased fluorescence intensity after 24 h in comparison with standard LNPs. Notably, the polymer-incorporated LNPs carrying a luciferase mRNA demonstrated a substantial increase (**~2 orders of magnitude**) in luciferase gene expression following intravenous (IV) and intramuscular (IM) injections, when compared to standard LNPs. Importantly, the polymer-incorporated LNPs displayed no *in vivo* toxicity when tested the for the serum levels of liver enzymes, including Alanine transaminase (ALT) and Aspartate transaminase (AST).

Conclusions

This study demonstrates that the polymer could be incorporated into various LNP formulations featuring different ionizable cationic lipids, yielding consistent and promising outcomes. The resulting formulation also serves as a proof of concept for incorporating similar class of polymers in traditional LNPs for improved transfection efficiency paving way for clinical translation.

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Abstract #3

Top-down optimization of lipid nanoparticles for high mRNA loading

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Background

When formulating messenger ribonucleotide acid (mRNA) into lipid nanoparticles (LNPs), there is a significant fraction of LNPs that are not loaded, namely, they are empty LNPs (eLNPs)[1]. The presence of eLNPs might be immunogenic and beneficial as adjuvants for vaccine applications[2] but very likely leads to unwanted side effects due to their reactogenicity.[3] Currently, there is no efficient approach to separate eLNPs and thus evaluate their role in the delivery of mRNA therapeutics.

Methods

In this study, we formulate firefly luciferase mRNA (mFluc) as reporter gene into our LNPs. Then, we use our proprietary method to separate LNPs of various mRNA loading. After separation, we investigate the therapeutic potency of LNPs of various mFluc loading both *in vitro* and *in vivo.*

Results

We show that our approach could efficiently separate LNPs of various mRNA loading. The structural characterization indicates that the method does not disturb the structural integrity of LNPs. Moreover, ribogreen assay shows that the mRNA encapsulation efficiency of the final products is over 95%. *In vitro* luciferase assay shows that the presence of eLNPs could significantly reduce the translation of mRNA by ten fold. The LNPs yielded by removing eLNPs show potential for better mRNA expression, especially at higher doses. The preliminary *in vivo* results show comparable expression between the LNPs with or without eLNP removal.

Conclusions

The top-down optimization method presented in this poster shows a highly efficient yet simple approach to separate LNPs based on their RNA loading state. It's a generic method for any LNP formulations. LNPs optimized with this method have the potential to deliver RNA therapeutics with improved translation and mitigated side effects.

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Abstract #4

Development of lipid nanoparticle formulations for *in vivo* brain delivery of nucleic acids

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Background

Gene therapy for the treatment of genetic neurological diseases requires the delivery of therapeutic agents to affected cells and regions of the brain. Because many current approaches to brain gene therapy (including antisense oligonucleotide therapeutics and gene delivery facilitated by viral vectors) are limited by functionality, potency, and safety, there is ample opportunity for the innovation of novel delivery systems for brain gene therapy drugs. Lipid nanoparticle (LNP)-mediated gene therapy, enabled by the same technology as the LNP-siRNA drug Onpattro™ and the Pfizer-BioNTech and Moderna COVID-19 vaccines, is a promising alternative modality for genetic brain disease treatment. The safety of LNP systems is well-established[1], and neurons are highly amenable to LNP transfection[2]. Using *EGFP* and luciferase reporter systems, we identified two LNP formulations (containing the ionizable cationic lipid and phospholipid combinations MC3 DOPG and DODMA DOPE) that efficiently and safely deliver siRNA and mRNA to mature primary neurons *ex vivo*. We hypothesized these formulations may also be suitable for *in vivo* use in the brain, and explored LNP distribution and activity in murine striatum following direct intraparenchymal LNP injection. We also assessed the correlation between *ex vivo* and *in vivo* activity of LNPs in the brain.

Methods

Ex vivo primary neurons were cultured from transgenic murine embryos, and day 7 *in vitro* mature neurons were treated with LNP formulations prepared with unique combinations of ionizable cationic lipids and phospholipids containing 0.01-1.0 µg/mL nucleic acid. Highly efficient candidate formulations containing 100-1000 µg/mL nucleic acid were injected into the striata of adult mice, and *in vivo* LNP distribution and activity were measured.

Results

Optimized LNP formulations significantly improved siRNA and mRNA delivery to mature primary neurons *ex vivo*. *In vivo,* direct intraparenchymal injection effectively delivered LNPs to murine striatum, and LNP distribution and activity in the brain were impacted by injection volume, nucleic acid dose, and LNP composition.

Conclusions

The efficacy and safety of optimized LNP compositions demonstrates the utility of these LNP systems as a research tool for studying genetic neurological disease. LNPs may also be an effective modality for therapeutic knockdown of gene products or gene replacement therapy in the brain *in vivo*.

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Abstract #5

Repurposing an Anticancer Agent for Fibrosis Therapy: Enhanced Oral Bioavailability of Sorafenib and Liver Fibrosis Mitigation through Modified Chitosan-Loaded Nanoparticles

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Background

Liver fibrosis, a consequential response to chronic liver injury, is characterized by excessive extracellular matrix (ECM) accumulation, notably collagen fibers. If untreated, fibrosis can progress to cirrhosis and hepatocellular carcinoma, increasing mortality risk. There are currently no FDA-approved drugs targeting liver fibrosis. Sorafenib, a small molecule tyrosine kinase inhibitor has been shown to inhibit hepatic stellate cell (HSC) activation and collagen deposition. However, adverse effects including cardiovascular, gastrointestinal, renal, and dermatological complications associated with its long-term usually result in therapy cessation. Enhancing Sorafenib's oral bioavailability can mitigate adverse effects by optimizing its pharmacokinetics, providing controlled release, and potentially allowing for lower effective doses, improving the drug's safety profile. This study used modified Quaternary Ammonium Palmitoyl Glycol Chitosan (GCPQ) to develop Sorafenib-loaded GCPQ nano micelles with enhanced bioavailability and sustained release profile.

Methods

A nano-micellar formulation of sorafenib was developed by probe sonication method utilizing a specialized polymeric carrier, GCPQ. Surface morphology, size, zeta potential, and encapsulation efficiency were determined. Invitro drug release studies were carried out throughout 48 to 56 hours. Invitro cell studies including cell migration assay, and scratch test were carried out to determine the effect of nanoformulation on the activation of HSC. Western blotting was done to check the expression of HSC activation-associated proteins including α-SMA, Collagen I, and Vimentin. Oral biodistribution and in-vivo pharmacokinetic studies were done in BALB-c mice.

Results

The optimized sorafenib-GCPQ nano micelles were spherical with a hydrodynamic particle size of < 70 ± 2.3 nm, a PDI of < 0.25, and a zeta potential of 35 ± 2 mV. A drug loading of > 85 % was achieved. An in-vivo pharmacokinetic study of the nanoformulation demonstrated a sustained release profile over 36 - 48 hours. The mean plasma concentration-time profile showed approximately 4-fold enhanced plasma concentration of sorafenib (C max: 28 ug/mL) in mice treated with Sorafenib-GCPQ as compared to the same dose of sorafenib suspension (C max: 8ug/mL), The same trend is reflected in Area under the curve calculations, confirming the enhancement of bioavailability for the nanoformulation. In-vitro, cell studies including migration assay and scratch assay indicated significant ($P < 0.05$) decreased activation of HSCs treated with sorafenib-GCPQ. Moreover, western blot showed the effect is mediated by modulation of the TGF-β1/Smad/EMT signaling pathway as shown by a decrease in the expression of vimentin, AKT, α-SMA, and Collagen I.

Conclusions

This study showcases the enhanced oral bioavailability and sustained release of Sorafenib via oral delivery employing GCPQ nanoparticles in mice. Encouragingly, *in vitro* studies of this formulation demonstrate promising outcomes in alleviating HSC activation, a significant indicator of liver fibrosis.

Abstract #6

Nanoparticle-Mediated Pyronaridine Delivery for Targeted ERCC1/XPF Inhibition and Sensitization of Head and Neck Cancer Cells to Cisplatin

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Background

Head and neck cancer (HNC) is the seventh most diagnosed cancer globally, accounting for 325,000 deaths annually[1]. Nonsurgical treatments for HNC mainly include radiation and platinum-based therapies. However, cancer cells' ability to repair DNA damage can undermine the effectiveness of these treatments. The HNC recurrence rate can be as high as 50% in treated patients. ERCC1-XPF is a key enzyme involved in the repair of DNA damage caused by chemo and radiotherapy used in HNC. High ERCC1 expression correlates negatively with clinical outcomes in HNC patients receiving cisplatin-based chemoradiotherapy for locally advanced disease [2]. Our research team has found a clinical anti-malarial drug, Pyronaridine (PYD), to inhibit ERCC1/XPF and sensitizing cancer calls to ionizing radiation (IR) [3]. This study aimed to investigate the synergistic activity of free versus liposomal formulations of PYD in combination with cisplatin in FaDu HNC cells, *in vitro*.

Methods

Liposomal PYD were prepared through ethanol injection method using acetate buffer aqueous phase (pH 3.5) followed by external buffer exchange with PBS and active loading of PYD. Prepared formulations were characterized for its size, encapsulation efficiency and *in vitro* release making comparisons with a similar formulation prepared by film hydration method. Cytotoxicity of cisplatin, PYD, or liposomal PYD as well as combination of cisplatin with PYD formulations against FaDu cells were investigated by MTT and colony formation assay. The synergistic effect between the combinations was assessed using Combenefit software.

Results

The IC_{50} of cisplatin was significantly reduced when combined with 0.5 and 1 μM of PYD in FaDu cells. Furthermore, Combenefit analysis showed a synergistic effect between cisplatin (5-10) μM and PYD (1.2-1.4) μM. Treatment of cells with a combination of cisplatin and liposomal PYD, led to a wider synergistic concentration range and an augmentation in the magnitude of synergy. The synergy between PYD formulations and cisplatin was also confirmed by colony formation assay.

Conclusions

The results revealed the potential of Pyronaridine as an effective ERCC1/XPF inhibitor, enhancing the sensitivity of HNC cells to cisplatin-based therapy. Liposomal delivery of PYD further enhanced the chemo-sensitizing activity of PYD in FaDu HNC cell line.

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Abstract #7

Nano-formulation of S4 and S4Br as novel competitive inhibitors of polynucleotide kinase/phosphatase (PNKP) in colorectal cancer therapy

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Background

Polynucleotide kinase/phosphatase (PNKP) is a bifunctional DNA repair enzyme which phosphorylates DNA 5′-termini and dephosphorylates DNA 3′-termini, making the damaged DNA termini amenable for ligation. PNKP inhibitors (PNKPi) can make cancer cells more sensitive to DNA damage by ionizing radiation or topoisomerase I inhibitors. Our team has identified S4 and S4Br as novel and more potent competitive PNKPi (IC_{50}= 170 and 640 nM, respectively) when compared with A83B4C63 (IC_{50} =2.4 μM), a previously identified non-competitive PNKPi [1]. We aimed to develop nanocarriers of S4 and S4Br and investigate their potential anticancer activity in colorectal cancer (CRC) cells.

Methods

Nano-formulations were prepared by dissolving S4 or S4Br and poly (ethylene oxide)-poly(caprolactone) (PEO-PCL) or poly (ethylene oxide)-poly (D, L-lactide) (PEO-PDLLA) in DMSO followed by dropwise addition of this solution to distilled water and dialysis against water. The prepared formulations were characterized for the level of encapsulated S4 or S4Br using UV/Vis spectroscopy at 440 nm and 445 nm, respectively, and average diameter using dynamic light scattering. Cytotoxicity of S4/S4Br was measured in wild type HCT116 (WT HCT116) and its Phosphatase and tensin homolog (PTEN) knock-out (HCT116 PTEN-/-) phenotype using MTT and colony forming assay.

Results

The average diameter of prepared nanoparticles was below 150 nm. Highest encapsulation efficiency (14.60 and 61.20 %) and loading content (4.10 and 5.57 %) for both S4 and S4Br was achieved in PEO-PDLLA micelles. The MTT assay showed higher IC_{50} for S4 and S4Br in WT HCT 116 (IC_{50}=1.19 and 50.35 μM, respectively) compared to HCT116 PTEN-/- cells (0.06 and 17.70 μM, respectively) indicating synthetic lethality. In comparison the IC_{50} of A83B4C63 in HCT116 $PTEN^{-/-}$ cells was reported at 5 μM [1]. Accordingly, clonogenic survival assay showed HCT116 /$PTEN^{-/-}$ to be more sensitive to S4 and S4Br at doses over 1.25 and 12.5 μM, respectively, while no toxicity in WT HCT116 at same concentrations.

Conclusions

Data confirms the anti-cancer activity of S4 and S4Br in PTEN negative CRC in line with what is expected from a PNKPi. S4 was shown to be a more potent PNKPi. Data also shows a good potential for PEO-PDLLA nanocarriers for solubilization and delivery of S4 and S4Br in CRC.

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Abstract #8

A Synergetic Approach Utilizing Nanotechnology, Chemotherapy, and Radiotherapy for Pancreatic Cancer Treatment

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Background

Pancreatic cancer is one of the leading causes of cancer deaths worldwide. Both Chemotherapy and radiotherapy (RT) suffer from normal tissue toxicity. To overcome this problem, we are proposing incorporating nanoparticles as radiosensitizers and as drug delivery vehicles into current chemoradiation regimes. Gold nanoparticles (GNPs) and Docetaxel (DTX) have shown very promising synergetic radiosensitization effects despite DTX toxicity to normal tissues. In this experiment, we explored the efficacy of the triple combination of DTX prodrug encapsulated in lipid nanoparticles (LNP_{DTX-P}), GNPs and RT in a 3D co-culture pancreatic cancer spheroid model.

Methods

The study utilized a co-culture spheroid model comprising MIA PaCa-2 cancer cells and patient-derived cancer-associated fibroblasts (CAF-98) to mimic pancreatic cancer conditions. The spheroids underwent treatment with GNPs (7.5 μg/mL of 13 nm in diameter functionalized with PEG and RGD peptide), LNP_{DTX-P} (99 nM of DTX prodrug), and 2 Gy of RT. GNP content in cells was measured using Inductively Coupled Plasma–Mass Spectrometry (ICP–MS). Cell viability was evaluated using the CellTiter-Glo 3D assay, while the assessment of DNA double-strand breaks (DSBs) was conducted by examining the expression of the DNA damage marker 53BP1 through an immunofluorescence assay.

Results

LNP_{DTX-P} treated 3D co-culture spheroids exhibited more cells in the G2/M phase and 183% more GNP compared to controls. Although GNPs/RT and RT/LNP_{DTX-P} showed a reduction in spheroid size and an increase in DNA DSB damage, the combination of the two nanoparticle radiosensitizers, GNPs, and LNP_{DTX-P}, with RT significantly enhanced the anti-cancer efficacy resulting in a 28% decrease in spheroid size and a 39% increase in DNA DSB.

Conclusions

The combination of GNPs and LNP $_{DTX-P}$ with RT showed a synergetic effect due to their radiosensitizing properties improving the therapeutic efficacy of each treatment modality alone. This triple modality offers a hopeful strategy to improve cancer treatment efficacy while reducing adverse effects.

Acknowledgements

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Abstract #9

Plasmonic enhanced femtosecond laser anticancer drug delivery using gold-lipid nanoparticles

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Background

Cancer spreads widely and is difficult to treat. Chemotherapy is widely used to treat cancer, however, as it is not specific to cancer cells, less than 5% of the administered dose target cancer cells. Our aim is to improve local treatment using tumour-specific light triggered release using liposomes (LNPs) containing photoreactive gold nanoparticles (AuNPs), and the chemotherapeutic agent, doxorubicin (DOX).

Methods

Using MDA-MB-231 breast cancer cell lines, proof-of-concept is performed with a femtosecond laser (800 nm wavelength, 55 fs pulse duration). The liposome formulation (100 nm) is formulated from DODAP/DSPC/Chol/PEG-DSPE (molar ratio 10/59/40/1) with encapsulated DOX (5 µg/mL) and 5 nm gold nanoparticles between the bilayers. Optimization of laser irradiation parameters led to optimal fluence (J/cm2) and pulses per spot for irradiating a 2D cell culture. A comparison was made between the effects of irradiation and LNPs-AuNPs-DOX formulation with the positive control (unencapsulated chemotherapy) and negative controls (irradiation with LNPs-AuNPs, LNPs-DOX and LNPs, and no irradiation on LNPs-AuNPs-DOX).

Results

Preliminary results have demonstrated proof-of-principle in a 2D breast cancer cell line model MDA-MB-231. Optimization of laser irradiation parameters resulted in an optimal fluence of ~100 mJ/cm^2 with ~2 pulses per spot, leading to a reduction in cell viability of up to 30% with the combination of lasers and the novel formulation LNPs-AuNPs-DOX compared to the effect of the laser alone on cells. Viability assays showed that the ultrafast laser did not affect cell viability with cell viability above 95% for the control group without LNPs-AuNPs-DOX for fluences below 250 mJ/cm^2. Also, viability assay shown that free DOX alone was expected to kill up to 60% of cells.

Conclusions

The method of treating cancer by combining irradiation and liposome formation increases both the specificity of chemotherapy delivery and the treatment. These results showed a significant increase in the therapeutic efficacy of the treatment in a 2D cell line model. Further development of studies in 3D models will enable similar results to be obtained.

Abstract #10

Increasing circulating lysosomal acid lipase in a mouse model of atherosclerosis using lipid nanoparticles

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Background

Atherosclerosis, manifested as ischemic vascular disease, is the leading cause of death globally. Atherosclerosis is characterized by accumulation of excess cholesterol in artery walls. The main cell type that takes up cholesterol in arteries, the smooth muscle cell [1], is deficient in a key enzyme, lysosomal acid lipase (LAL), that is required for cholesterol metabolism. Cholesterol-loaded smooth muscle cells increase their efflux of excess cholesterol when treated with recombinant LAL protein *in vitro* [2], and intravenous injection of recombinant LAL protein has been shown to reduce atherosclerosis in mice [3]. We hypothesized that delivery of LIPA (LAL gene) mRNA to the liver using lipid nanoparticles would increase circulating and aortic LAL in a mouse model of atherosclerosis.

Methods

Lipid nanoparticles (LNP) formulated with LIPA or Luciferase mRNA were injected intravenously or intraperitoneally into ApoE-deficient or AAV-PCSK9 C57BL/6 (partial LDLR-deficiency model) mice at 1 or 2 mg/kg and serum LAL activity measured post-injection. Mouse survival after 6 weekly injections was also determined.

Results

LNP-1 were formulated using codon-optimized and fully N1 methyl pseuroduridine substituted mRNA, with lipids including Acuitas proprietary lipid [4]. Particle size was 80 nm +/- 5, PdI < 0.2, and encapsulation > 95%. Intravenous (IV) injection of LIPA LNP-1 at 2 mg/kg resulted in a 3.1-fold and 1.7-fold increase in mouse serum LAL activity from baseline 6 hrs after injection in male and female ApoE-deficient mice, respectively. Mortality after 6 weekly IV injections was 100% in males and 50% in females, with a trend toward reduced aortic root lesion area in surviving mice. Intraperitoneal (IP) injection also increased LAL activity, and reduced mortality in females but not males. IV dosing at 1 mg/kg reduced mortality but did not produce increased serum LAL. NanoVation proprietary long-circulating LNP were tested in AAV-PCSK9 mice and showed improved survival but did not increase serum LAL by IV or IP routes. LNP-2 (similar to LNP-1 but using SM-102 lipid) dosed at 1 mg/kg IP in AAV-PCSK9 mice increased LAL activity by 1.3-fold at 24 hrs.

Conclusions

Increasing LAL using LNPs may reduce atherosclerosis, but toxicity after multiple doses in ApoE-deficient mice is prohibitive: utilizing the AAV-PCSK9 model may be a viable alternative. Further investigation is needed to explore the observed sex differences in toxicity and serum LAL. Long circulating LNPs showed reduced toxicity but failed to increase serum LAL, likely due to lower liver expression. The IP route appears to be an effective and technically easier alternative to IV for the tested formulations. Despite loss of LDLR in AAV-PCSK9 mice, LIPA mRNA was expressed and LAL activity detected in mouse serum after LNP-2 injection.

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Abstract #11

Long-term gene editing following intramuscular injection of LNP-encapsulated base editors

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Background

Base editors have the potential to permanently change a single base in the genome, providing the potential to address the root cause of up to 68% of monogenic diseases[1]. The edit is presumably permanent; however, for edited tissues to persist in the body they must continue to proliferate and not be attacked by immune response. To date, long-term gene editing following intramuscular injection of LNP-encapsulated base editors has yet to be explored.

Methods

We used a transgenic mouse model which enables the live detection of base editing. The model harbors a luciferase gene with an ABE-correctible premature stop codon, producing luminescence in areas that have been successfully edited. We injected mice intramuscularly in one hindlimb with two clinically approved LNP formulations. LNPs were made with molar ratios of 50% ionizable lipid (either Dlin-MC3-DMA or ALC-0315), 10% DSPC, 38.5% cholesterol and 1.5% PEG-DMG. Following injections, mice were regularly imaged for luminescence. After 72 weeks a terminal luciferase assay and sequencing was performed on extracted tissues.

Results

Gene editing persisted for 72 weeks following intramuscular injection. Editing was localized to the hind limb in formulations containing DLin-MC3-DMA, while those with ALC-0315 showed editing in the hind-limb and liver.

Conclusions

In this study, we confirmed that LNPs can be used to deliver gene editing components intramuscularly for permanent, long-term expression of corrected genes. This has the potential to impact diseases affecting proteins produced in the muscle.

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Abstract #12

Cell penetrating peptide modified LNPs delivering R848 cures and immunizes animals with peritoneal carcinomatosis

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Background

Peritoneal carcinomatosis (PC) is a late-stage cancer that has a high recurrence rate and mortality after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy due to incomplete elimination of cancer.

Methods

To improve the existing standard of care for PC, we developed a lipid nanoparticle formulation modified with a cell penetrating peptide (CPP-LNP) that localizes a toll-like receptor agonist, resiquimod (R848), in the peritoneal cavity to activate the immune system for cancer therapy.

Results

CPP-LNP increased R848 retention in the peritoneum by 26-fold and increased R848 uptake by the peritoneal immune cells by 48-fold compared to free R848. Additionally, CPP-LNP increased the uptake of model antigen by dendritic cells, leading to strong cytosolic release. These findings resulted in a 2-fold increase in IFN-α induction in the peritoneal fluid, without elevating plasma levels. In a CT26 colon cancer model with peritoneal metastases, CPP-R848 combined with oxaliplatin, an immunogenic cell death inducer used in the chemotherapy, completely eliminated the metastases in 70% of the mice. These mice survived beyond 120 days and were cured, while the control mice reached humane endpoints by 30 days. The cured mice developed specific antitumor immunity as no tumor development was observed when rechallenged with the same tumor cells. However, inoculation of a different tumor line resulted in tumor growth. Increased T cell populations and cytokine showed long term immune memory upon exposure to specific tumor. We also confirmed the importance of different immune populations in this immunotherapy using immune depletion models.

Conclusion

In summary, CPP-R848 is a promising new immunotherapy for peritoneal metastases of cancers. It improves the retention and uptake of R848 by peritoneal immune cells and enhances the induction of IFN-α in the peritoneal fluid. Combining CPP-R848 with oxaliplatin induces immunogenic cell death, leading to complete elimination of metastases and the development of specific antitumor immunity.

Abstract #13

Tri-Component Polyplexes for Enhanced Delivery of Self-Amplifying RNA

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Background

Self-amplifying RNA (saRNA) is a next-generation vaccine platform, which requires a smaller dose than mRNA to induce an immune response and could reduce side effects and increase vaccine production efficiency. Like other RNA vaccines, it requires a delivery system to prevent degradation and promote cellular uptake. Many delivery systems already exist, including lipid nanoparticles (LNPs) and cationic polymers, such as pABOL, a polymer which has been optimized for delivery of saRNA. Comparisons of LNPs and pABOL saRNA vaccine delivery have shown that LNP delivery led to an overall higher immune response, while pABOL delivery induced approximately 100x higher intramuscular protein expression [1]. These delivery systems differed mainly in their surface charge, and it has been shown that surface charge greatly impacts cellular tropism [2]. Using biocompatible anionic polymers to neutralize pABOL polyplexes high surface charge, we aim to alter the cellular tropism and increase the immunogenicity while maintaining high protein expression of pABOL polyplexes to yield highly efficient vaccine delivery systems.

Methods

Anionic polymers were chosen based on biocompatibility, accessibility, and previous use in therapeutic applications. Anionic polymers will be incorporated via direct addition at varying weight to weight ratios to pABOL-saRNA polyplexes. Particle characteristics (size, polydispersity index and surface charge) are then characterized via dynamic light scattering. Transfection efficiency is evaluated by transfecting HEK 293T cells in-vitro and performing luciferase assays.

Results

We have identified a polymer that has successfully been formulated with pABOL-saRNA polyplexes (anionic polymer 1 (AP1)) to create formulations with surface charges ranging from +15mV to -20mV, with all triplexes maintaining an identical level of in-vitro luciferase expression (100,000 RLU in 50,000 cells) to pABOL-saRNA controls. Additionally, AP3 was able to significantly increase protein expression of polyplexes in-vitro. Other anionic polymers (AP2) were detrimental to polyplexes transfection efficiencies at higher weight to weight incorporations.

Conclusions

The identity of the anionic polymer used has been shown to impact the resulting tri-complexes ability to transfect cells in-vitro. It has also been shown that the molecular weight of the anionic polymer impacts transfection efficiency, with lower molecular weight anionic polymers being favoured.

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Abstract #14

A novel method to extract nanoparticles from complex biological media

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Background

The limited understanding of the interactions between nanoparticles and their biological environment hampers the development of nanomedicines.[1, 2] Adsorbed proteins on nanoparticles influence their distribution *in vivo*.[3] To probe these interactions, nanoparticles and their protein corona must be extracted from biological environments. This is a challenging task for liposomes and lipid nanoparticles (LNPs) due to their low density. Understanding the protein corona that deposits on the surface of nanomedicines therefore requires tedious optimization and controls.

Methods

We are developing a gentler nanoparticle extraction method that does not require high-speed centrifugation and is robust enough to work in complex biological environments. This method should make it possible to isolate liposomes, LNPs or polymer nanoparticles to characterize their protein corona, but also to evaluate their drug content and other properties.

Results

First, we prepared radiolabeled LNPs with three ionizable lipids (DODAP, DLin-MC3-DMA and SM-102). We showed that the immunoprecipitation developed before in our laboratory (using magnetic beads and anti-PEG antibodies) is poorly amenable to the extraction of lipid nanoparticles. We evidenced non-specific interactions between LNPs and the magnetic beads. To explore our novel approach, we incubated radiolabeled NPs with a proprietary scavenging molecule and collected them using minimal centrifugation (100 *g*). As a preliminary result, we successfully extracted radiolabeled pegylated liposomes from PBS.

Conclusion

This new method acts as a proof of concept in the development of more selective, softer and faster ways to isolate nanoparticles. We will continue exploring whether this method is adequate to extract LNPs from biological media. We believe that this new tool could help in probing the interactions of nanoparticles with proteins, while maintaining their structural integrity.

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Abstract #15

Novel Polyethyleneimine Derivatives for Gene Silencing in Cancer

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Background

RNA interference (RNAi) holds great promise for treating various diseases, yet its clinical application faces challenges due to the need for effective and safe delivery systems. Cationic polyethyleneimine (PEI) derivatives have emerged as attractive nonviral gene carriers in gene therapy.

Method

This study aimed to develop and characterize a novel siRNA delivery system, utilizing α-benzyl carboxylate-ε-caprolactone (BCL)-modified low molecular weight polyethyleneimine (PEI-BCL) to enhance siRNA transfection while minimizing cytotoxicity. The synthesis of PEI-BCL involved various Ethyleneimine:BCL molar ratios (1:0.5, 1:1, and 1:10) through the ring-opening of BCL by PEI in the presence of Stannous Octoate as a catalyst. Prepared polymers were characterized by ^{1}HNMR, ^{13}CNMR, and FTIR. Polyplexes were prepared at various polymer/siRNA ratios and characterized for average diameter, polydispersity (PDI) and ζ-potential. SiRNA binding was quantified using the Sybr Gold assay. Transfection efficiency was assessed in three cancer cell lines MDA-MB231, H1299, and H1975 GFP-positive cells, utilizing GFP or scrambled siRNA, making comparisons with Lipofectamine RNAiMAX, Primefect, and PEI2k complexes of siRNA.

Results

The successful synthesis of PEI-BCL was confirmed with increasing mole % BCL to total NH2 conjugation across the applied 1:0.5, 1:1, and 1:10 ratios. Polyplexes displayed a compact size, ranging from 130-150 nm, with an acceptable polydispersity index (PDI). The ζ-potential of polyplexes in water measured around 43 ± 0.63 mV. Notably, PEI-BCL formulations at 1:1 and 1:10 PEI:BCL ratios demonstrated superior transfection efficiency compared to PEI2k. Furthermore, they exhibited lower cytotoxicity (polymer/siRNA ratio 2.5) than commonly used transfection agents, such as Lipofectamine RNAiMAX and Primefect. The highest GFP silencing efficiencies were observed with PEI-BCL (1-10), reaching 66% in MDA-MB-231 GFP+ cells and 40% in H1299 GFP+ cells at a polymer-siRNA ratio of 2.5. In H1975 GFP+ cells, a substantial 45% silencing was achieved at a polymer-siRNA ratio of 10, with no significant observed toxicity.

Conclusion

Our study presents a novel siRNA delivery platform, PEI-BCL, with superior transfection efficiency compared to PEI and reduced cytotoxicity compared to conventional siRNA transfecting agents. PEI-BCL demonstrates potential as a promising nonviral gene delivery vector, addressing the critical need for safe and efficient siRNA delivery.

Abstract #16

A liposomal formulation containing disulfiram and its anti-cancer metabolite for activation of ROS and anti-tumour immunity

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Background

Disulfiram was approved by the FDA for the treatment of alcohol abuse under the brand name Antabuse in 1951. However, for over 40 years there has been evidence supporting disulfiram's use as an anti-cancer drug. Upon administration, disulfiram is hydrolyzed to form two molecules of DDC, which upon binding Cu forms $Cu(DDC)_2$, the proposed active form against cancer. We have developed $Cu(DDC)_2$ liposomal formulations which were demonstrated to activate anti-cancer immunity in a vaccination model, although the relative response was limited. It was observed that when disulfiram and DDC bind endogenous Cu in the cell there is generation of reactive oxygen species (ROS), which is linked to the induction of immunogenic cell death (ICD), while pre-forming $Cu(DDC)_2$ in our liposomes led to an inhibition of ROS. We sought to modify our $Cu(DDC)_2$ formulation to include the ROS-generating properties of disulfiram to potentially generate a more robust immune response.

Methods

A cancer vaccination model of anti-tumour immune response was used to characterize anti-tumour immune response of the initial $Cu(DDC)_2$ formulation. Cytotoxicity and ROS were detected with the IN Cell Analyzer 2200, and cell-surface calreticulin, and extracellular HMBG1 and ATP were detected in mouse colorectal cancer cells (CT26) as markers of ICD. Disulfiram-infused $Cu(DDC)_2$ ($diCu(DDC)_2$) ROS-inducing liposomes were formed by dissolving 2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol (CHOL), and disulfiram in chloroform in various molar ratios using the thin film hydration method. DDC and $CuSO_4$ were added sequentially to the liposomes forming $Cu(DDC)_2$, which was shuttled inside the liposomes due to DDC's ionophore ability. Cytotoxicity and ROS were detected in CT26 cells treated with the resulting formulations.

Results

The ratio of 50:40:10 DSPC/Chol/disulfiram was found to be optimal for extrusion, and the resulting liposomes were approximately 100 nm with 0.1 polydispersity. Disulfiram in liposomes did not hydrolyze into DDC upon $CuSO_4$ addition, demonstrating its stability for $Cu(DDC)_2$ loading, and empty disulfiram liposomes were cytotoxic in CT26 cells prior to $Cu(DDC)_2$ loading. The $diCu(DDC)_2$ liposomes had comparable $Cu(DDC)_2$ levels to the previous disulfiram-free $Cu(DDC)_2$ formulation indicating that the presence of disulfiram did not inhibit loading, and while the prior formulation inhibited ROS, the $diCu(DDC)_2$ liposomes resulted in a 3-4 fold increase in ROS generation.

Conclusions

This study describes a modification of liposomal $Cu(DDC)_2$ to include the parent compound disulfiram in the lipid bilayer. The resulting formulation maintains cytotoxicity while enhancing ROS, and studies are ongoing to examine the co-delivery of these components to improve the induction of ICD and anti-tumour immunity *in vivo*.

Abstract #17

Gene Correction in Humanized Mutant Progranulin Mice for the Treatment of Progranulin-Associated Frontotemporal Dementia

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Background

Loss of function mutations in the progranulin (*GRN*) gene are a prevalent etiological factor in familial frontotemporal dementia (FTD)[1]. There are currently no approved therapies for FTD stemming from GRN deficiency. An ideal therapeutic strategy would seek to restore GRN expression by correcting the deleterious mutations in affected patients.

Methods

CRISPR/Cas9-mediated gene editing is a novel approach to targeting monogenic hereditary disorders[2] such as *GRN*-dependent FTD. To develop a CRISPR-mediated gene therapy drug, we employ a 'humanized' mutant murine model of FTD developed recently in our laboratory. Our existing murine model that expresses a copy of the human *GRN* gene in the absence of murine *Grn*[3] has been further bioengineered to carry a common pathogenic variant that results in the absence of GRN production. We deliver CRISPR/Cas9 as the ribonucleoprotein (RNP) complex co-administered with guide-RNAs and DNA repair templates via a novel lipid nanoparticle (LNP) formulation developed by our industry partners at Incisive Genetics. This approach induces homology-directed repair (HDR) at the mutant *GRN* locus to restore GRN expression. Leveraging the liver's inherent affinity for LNPs[4,5], we evaluate drug delivery and on-target genome editing efficiency *in vivo* by intravenous administration of the drug followed by the assessment of HDR-mediated correction in hepatic cells.

Results

In a preliminary investigation involving an array of guide-RNAs and DNA repair templates, we conducted immunohistochemistry staining using a primary antibody against GRN to assess treated versus control animals' liver sections. These initial results demonstrate successful GRN expression restoration in select liver cells. Ongoing studies encompass editing quantification and dosage-dependent studies to enhance *GRN* correction in hepatic tissue.

Conclusions

We have demonstrated *in vivo* targeting of a common pathogenic variant of *GRN* in a relevant murine model system. Through optimizing our *GRN* targeting and HDR approach in the liver, our strategy will be employed in intra-striatal injections of the drug to facilitate the evaluation of *GRN* correction within brain cells. This project serves as a proof-of-concept study for the efficacy of LNP-CRISPR gene correction within the brain, specifically for the treatment of hereditary neurological disorders. Furthermore, it signifies the advancement of this targeted genome editing approach for FTD patients.

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Abstract #18

Specific light triggered techniques to insert siRNA in retinal cells

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Background

Age-related macular degeneration is a retinal disease involving blindness with a prevalence of 170 million people worldwide in 2016. Gene therapy is under study to improve the treatments by downregulating the vascular endothelial growth factor (VEGF), a protein at the origin of the disease. We are developing two light triggered systems to release siRNA. The first method is optoporation, an approach based on the interaction between laser irradiation and gold nanoparticles (AuNPs) to perforate the cell membrane to let the siRNA in [1, 2]. The second one is photoreactive lipid nanoparticles (LNPs) containing siRNA and 5nm AuNPs that open through irradiation thanks to the AuNPs. The great advantage of those techniques is the targeting: only irradiated cells for the LNPs, only targeted by the functionalized AuNPs and irradiated cells for optoporation, will be treated.

Methods

Using a femtosecond laser at 800 nm, optoporation was performed *in vitro* to insert propidium iodide (PI) and Cy3-siRNA in ARPE-19 cells. The efficiency is determined as the ratio of fluorescent cells over irradiated cells. The viability is determined as the ratio of dead cells after 24h over irradiated cells. For the photoreactive LNPs with Luc-siRNA, 22Rv1-Luc cells after a short incubation period with LNPs were irradiated, and the downregulation of luciferase measured by luminescence and viability with a Presto Blue assay 24h after treatment.

Results

Efficiency above 30% for PI and slightly above 20% for Cy3-siRNA has been reached while keeping the viability above 80% at all times for optoporation (Figure 1 a-b). In parallel, we showed an increase of the downregulation by 20% without irradiation to 40% with irradiation at 50 mJ/cm² while keeping the viability above 80% at all times (Figure 1 c-d).

Conclusions

Here, we prove that optoporation and the LNP system are both adequate techniques to deliver siRNA into cells. Next step will be to improve the LNP formulation to reduce the siRNA leakage without irradiation and to use siRNA targeting VEGF in order to quantify the induced downregulation of the gene before translating *in vivo*.

Acknowledgment

We thank Nanocore for their help and the development of the photoreactive LNPs.

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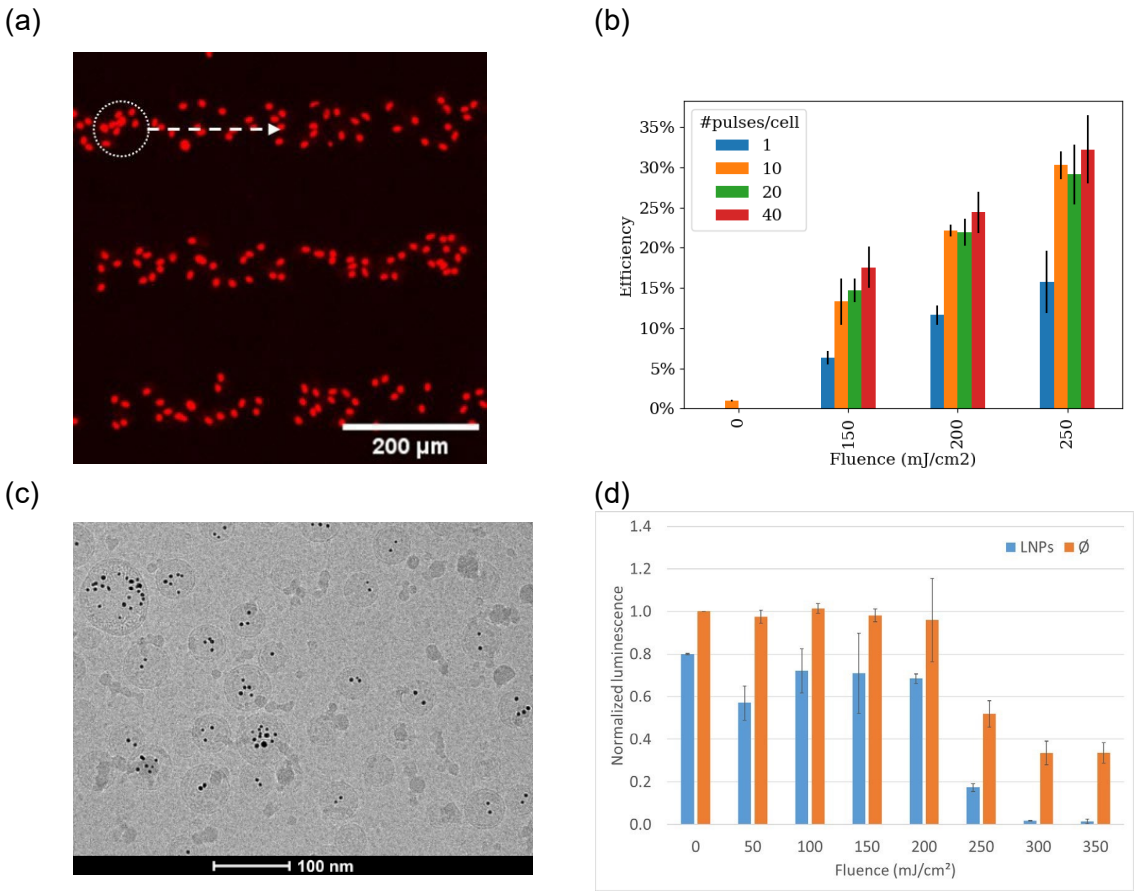

Figure 1. (a) *In vitro* ARPE-19 cells internalization of propidium iodide, 20min after irradiation with anti-CD44-PEG-NPs, 10^9 NPs/mL for the incubation, 250 mJ/cm², 10 pulses/cell, 80 µm beam diameter. The circle represents the spot size of the laser, the arrow is pointing the direction of irradiation. Cells were irradiated line by line spaced by 200 µm. Scale bar: 200µm. (b) Efficiency of the optoporation at difference fluences depending on the number of pulses received by each cell. (c) Cryo-TEM image of LNP-GNP-siRNA particles developed using the Onpattro-like formulation. (d) Luciferase luminescence normalized with LNPs (blue) and without (orange) at different fluence.

Abstract #20

Optimization and Delivery of Endogenous mRNA UTRs using LNPs to Control Exogenous Protein Expression in Platelets

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Background

Platelets are frequently transfused to control hemorrhage in trauma and surgery. Due to limitations such as variable donor-based hemostatic efficacy and decreased unit efficacy during hemorrhage, platelet transfusions are not always effective. To overcome these issues, we propose manipulating donor platelets through gene therapy. We have developed a method to deliver mRNA into donor platelets *ex vivo* using lipid nanoparticles (LNP), enabling exogenous protein expression. Despite successful LNP uptake and mRNA translation, exogenous protein synthesis levels remain low. To try and improve protein synthesis, mRNA sequences were modified in the untranslated region (UTR) upstream of the coding sequence. The 5' UTR possesses structural features that can influence ribosomal binding and control overall protein synthesis. The goal of this study is to increase exogenous protein synthesis in donor platelets *ex vivo*, through optimization of the LNP-delivered mRNA sequences to mimic highly expressed platelet proteins (Figure 1A).

Methods

mRNA sequences were designed to contain 5' UTRs from endogenous platelet proteins, and a coding sequence for the reporter luciferase. Initial mRNA screens were completed using LNP delivery to hepatocyte and megakaryoblast cell lines to determine if differences in protein synthesis were cell specific. mRNAs were then delivered using LNPs to pooled-donor platelets (obtained from Canadian Blood Services). Protein expression in all cells was determined using the luciferase assay.

Results

Delivered mRNAs containing platelet endogenous UTRs showed platelet-specific enhancement of protein expression compared to other cell types. The best performing UTR induced a 9.2-fold increase in expression over the lowest expressing UTR, and a 1.6-fold increase in expression over a standard UTR (Figure 1B).

Conclusions

These experiments show for the first time the influence of UTRs on protein synthesis in platelet cells. Additionally, through modification of the delivered mRNA UTRs, it is possible to increase protein expression. This study was supported by the US Department of Defense Investigator-Initiated Research Award.

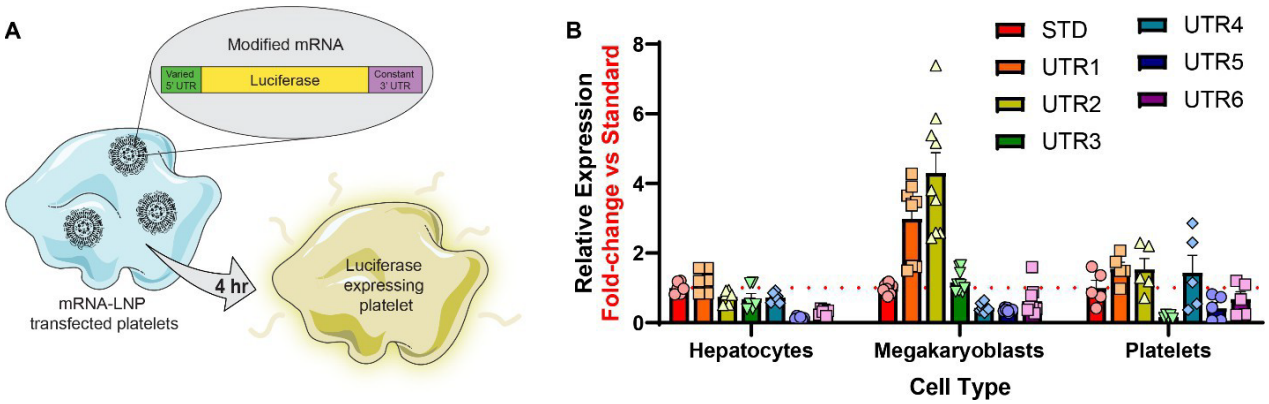

Figure 1 A) Platelets were transfected with lipid nanoparticles (LNP) containing optimized mRNA to enable expression of the exogenous protein luciferase. mRNA sequences were designed to mimic the untranslated region of highly expressed platelet proteins and mRNA, and contain the coding sequence for the reporter luciferase. B) Relative luminescence fold-change for each endogenous UTR versus a standard UTR for platelets (n=5, biological replicates), megakaryoblasts (n=3, biological replicates) and hepatocytes (n=3, biological replicates). Data reported as mean ± SEM.

Abstract #21

The effect of the self-assembly conditions on physicochemical characteristics of PEO-PBCL micelles for scale-up purposes

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Background

The use of block co-polymer based nanomedicine has attracted much attention in recent years. However, there are challenges associated with its application in clinical settings including scale-up issues [1,2]. The long-term objective of this study is to develop a process for scaled-up preparation of polymer-based nanomedicine. In this project, we assessed the effect of self-assembly conditions such as polymer concentration, organic solvent volume, and organic to aqueous media ratio on the physicochemical properties of poly(ethylene oxide)-poly (α-benzyl carboxylate-ε-caprolactone) (PEO-PBCL) nanoparticles, i.e, particle size and polydispersity, prepared at different scales between 0.6 to 5 mL.

Methods

PEO-PBCL was synthesized by ring-opening polymerization of α-benzyl carboxylate-ε-caprolatone(BCL), using PEO 5000 Da as initiator, stannous octoate as catalyst and dry toluene as solvent [3]. By using Nuclear Magnetic Resonance (NMR) spectroscopy, the degree of polymerization (DP) of BCL was quantified. Thin-layer chromatography (TLC) using ethyl acetate and hexane with 3:7 volume ratio as mobile phase assessed the purity of polymers from BCL. Block copolymers were self-assembled into nanostructures by a co-solvent evaporation method using acetone as the organic co-solvent while keeping the final concentration of polymer in the solution at 50-100 mg/mL. The level of acetone, acetone to water, and polymer concentration was changed during the self-assembly. After acetone evaporation, micellar size polydispersity index (PDI), and morphology were assessed.

Results

^{1}H NMR results indicated a DP of 10 for the PBCL segment of PEO-PBCL, while TLC showed the absence of free BCL. The best micellar size was observed when water was added to acetone at an acetone to water volume ratio of 1:1 with a polymer concentration of 50 mg/mL. The micellar size remained ~340nm when the micellar production volume was increased ~8 times more (from 600µL to 5000µL). Also, the PDI remained the same during the scaling-up process. Filtration of polymeric micelles using a 0.45µm sterile filter did not affect the micellar concentration.

Conclusion

The reported PEO-PBCL micelle preparation is a promising method for scaled-up production of these nanoparticles as it does not require many complicated steps to follow. Besides, the micellar diameter is independent of the size of the production batch.

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Abstract #22

Targeting ligands on an equilibrated nanoparticle protein corona enable cell targeting in serum

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Background

Targeting ligands are conjugated onto nanoparticles to increase their selectivity for diseased cells. However, they become covered by serum proteins which prevent them from binding to target cells.

Methods

We measured the thickness of the protein corona on nanoparticles using dynamic light scattering and quantified the protein adsorption and desorption rates using biolayer interferometry. We used molecular dynamics simulations to study the forces between proteins on the nanoparticle surface. We attached targeting ligands to the equilibrated protein corona of nanoparticles using click chemistry and measured cancer cell uptake of nanoparticles with inductively coupled plasma mass spectrometry and flow cytometry.

Results

Here, we show that the nanoparticle protein corona achieved a maximum thickness in serum because the protein adsorption and desorption rates reached an equilibrium. Simulation experiments suggest that the number of molecular interactions between proteins decrease with distance from the nanoparticle surface until the forces are too weak to hold the proteins together. This results in an equilibration state between the proteins on the nanoparticle surface and in biological fluids. Conjugating targeting ligands to this equilibrated protein corona allowed the nanoparticles to bind to target cells in the presence of serum proteins. In contrast, conjugating targeting ligands directly to the nanoparticle surface resulted in a 55% reduction in binding to target cells in serum. We demonstrated this concept using two nanoparticle material types with different surface chemistries (Figure 1).

Conclusions

We present a ligand-on-corona conjugation strategy that overcomes the negative impact of serum protein adsorption on nanoparticle cellular targeting.

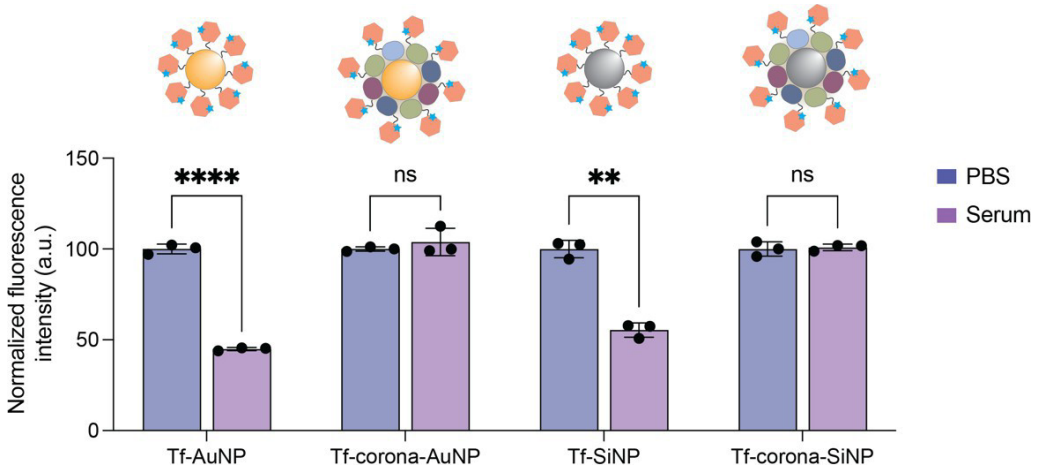

Figure 1: Ligand-functionalized equilibrated protein corona nanoparticles retain their targeting abilities in serum. Nanoparticle binding to target (SK-BR-3) cells is compared in phosphate buffered saline (PBS) and fetal bovine serum (Serum). Cartoon nanoparticle design schematics are displayed above the data. Gold and silica nanoparticles (Tf-PEG-AuNP and Tf-PEG-SiNP) with direct-conjugated transferrin show a significant reduction in binding in serum versus in PBS. When transferrin is conjugated to the equilibrated protein corona of gold nanoparticles (Tf-corona-AuNP) or silica nanoparticles (Tf-corona-SiNP), the targeting ability is retained. Data are shown as mean ± s.d. (n = 3). **** indicates $P < 0.00005$, ** indicates $P < 0.005$.

Abstract #23

Lipid Nanoparticles Mediate Promising CRISPR-based Gene Editing in Human Lungs

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Background

Cystic fibrosis (CF), the most common fatal genetic disorder in Canadian children and young adults, is caused by single mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [1, 2].Gene editing via CRISPR-based tools is potentially an unprecedented corrective therapy for a monogenetic disease like CF [3]. Yet currently there is no efficient way of delivering CRISPR-Cas9 through the lung epithelium and the protective mucosal layer lining it [4]. Lipid nanoparticles (LNPs) are currently the most promising non-viral vehicles for the delivery of nucleic acids [5, 6]. This study leveraged optimized Cas9 mRNA/sgRNA loaded LNPs to achieve promising gene editing levels in healthy and CF lung cells and physiologically relevant 3D models.

Methods

Transfection efficiency of different formulations of LNPs carrying GFP encoding mRNA (LNP-eGFP) was assessed in primary human bronchial epithelial (HBE) cells. Then, the gene editing capacity of LNP-CRISPR-Cas9 mRNA/sgRNA, was measured in HBE cells, normal human lung fibroblast cells (NHL-FB), lung endothelial cells (EC) and primary bronchial cystic fibrosis (BCF) cells. Bronchial epithelial models cultured at the air liquid interface were generated and differentiated to produce relevant lung tissue layers including mucus, cilia and lung epithelium. Healthy and CF lung models were then treated with promising LNP formulations and were further optimized by sgRNA modification or increasing sgRNA ratio to Cas9 mRNA.

Results

One LNP formulation (LNP-H) showed high gene editing ~35% in lung HBE, NHL-FB and EC cells. Further optimized LNP-H formulations employing highly modified sgRNA or 3:1 ratio of sgRNA to Cas9 mRNA, led to ~50% and ~30% editing, respectively, in BCF cells. LNP-H showed high uptake in both healthy and CF lung models as shown by florescence microscopy. Finally, in both CF and healthy lung cell models the gene editing was lower, likely due to protective lung barriers such as mucus and cilia, yet LNP-H was able to achieve ~8% in healthy lung models which was higher than in CF lung models (4-6%).

Conclusions

An LNP formulation (LNP-H) was able to achieve robust editing in primary HBE cells (~35%). Increasing the ratio of sgRNA to Cas9 mRNA to 3:1 in LNP-H resulted in ~50% editing in BCF cells. In both healthy and CF models, LNP-H showed high uptake while achieving around 8% gene editing in healthy models and 4-6% editing in CF models. Reduced editing in lung models generally and especially CF models is not unexpected due to the ciliated epithelium/mucosal barrier. With reports [7] indicating that relatively low levels (~10%) of normal CFTR mRNA are sufficient for normal lung function, this study presents promising LNP-mediated approaches for achieving effective CRISPR-based editing as a therapy for CF.

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Abstract #24

Histological evaluation of systemic nanoparticle distribution in tumour-draining cervical lymph nodes of orthotopic oral cavity cancer models

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Background

Cervical lymph nodes are often the first site of spread from oral cavity cancers, which necessitates their routine removal (dissection) during surgery. Typically, between 10~30 cervical nodes are dissected or biopsied [1] leading to complications such as lymphedema. Efforts to develop intra-operative tools that distinguish only metastatic nodes for dissection have focused on using fluorescent nanoparticles whose uptake in metastatic nodes is widely assumed to be driven by the cancer cells themselves. Herein, we evaluate the histological distribution of systemically administered fluorescent Porphysome (PS) nanoparticles in healthy and oral tumour-draining cervical lymph nodes to shed light on the mechanisms of nanoparticle uptake in lymph node metastases.

Methods

Two orthoptic models of oral cavity cancer were established using murine MOC2 oral squamous cells implanted in the tongues of immunodeficient RNU nude or immunocompetent F344 rats. Healthy rats served as experimental controls. Radiolabelled PS (10^{13} particles/kg, 200 MBq ^{64}Cu/kg) were administered IV bolus. 24 hours post-injection, the primary tumour and cervical nodes were excised, and flash frozen in OCT medium. Serial tissues sections were cut and stained for H&E, pan-Cytokeratin, CD3 (T and NK cells) and CD19 (B and follicular cells) for lymph node histopathological staging. Fluorescence microscopy and tissue autoradiography were performed to identify the intra-nodal distribution of PS in relation to metastatic deposits versus normal lymphoid tissues.

Results

54 cervical lymph nodes from healthy and tumour-bearing rats were analysed in this study. PS nanoparticle uptake was detected with fluorescence and autoradiography in 79% and 88% of healthy and metastatic lymph nodes, respectively. PS distributed primarily in the cortical region of the healthy and metastatic nodes, and preferentially in B cell-rich follicles. This distribution pattern was consistent between immune competent and compromised models. Most surprising, there was limited evidence of direct PS uptake by cancer cells and metastatic nests in the lymph nodes.

Conclusions

We report that cancer cells and metastatic nests in tumour-draining lymph nodes did not appear to directly uptake systemically administered PS nanoparticles. This suggests that if there is any intrinsic specificity of nanoparticles for lymph node metastases, a more complex explanation for their uptake is required.

Acknowledgements

This work was supported by the Canadian Cancer Society and the Terry Fox Research Institute.

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Abstract #25

Optimizing Self-Amplifying RNA Therapeutics: Impact of Delivery Systems and Administration Routes

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Background

The emergence of COVID-19 mRNA vaccines has underscored the clinical potential of RNA technology. Within this realm, self-amplifying RNA (saRNA) stands out as an innovative approach derived from alphaviruses. In contrast to traditional mRNA, saRNA encodes alphaviral non-structural proteins, enabling continuous replication in the host cytosol. This unique auto-replicative feature results in heightened and sustained protein expression with minimal doses, significantly alleviating manufacturing challenges during pandemics[1, 2]. While the therapeutic potential of mRNA has been extensively explored in terms of expression kinetics[3], immunogenicity[4], and delivery systems[5, 6], the distinctive attributes of saRNA remain largely unexplored.

Approach AND Methodology

This study investigates the impact of diverse administration routes on saRNA expression trajectories, employing advanced lipid nanoparticles (LNPs) and a cationic polymer (pABOL) for delivery. The research specifically focuses on intravenous (IV), intramuscular (IM), intraperitoneal (IP), intradermal (ID), subcutaneous (SC), and intranasal (IN) routes, the research investigate the biodistribution, protein expression levels, duration, and immunogenicity of saRNA. The primary objective is to provide crucial data for selecting optimal delivery routes and systems, applicable to therapeutic contexts like protein replacement therapy for cancer therapy or vaccines for infectious diseases.

Results

Administration of saRNA-LNPs encoding firefly luciferase (reporter protein) through various routes in female BALB/c mice unveiled distinct expression kinetics. The subcutaneous (SC) route exhibited the highest luciferase expression by day 2 but experienced a rapid decline by day 6. In contrast, IM, ID, and IP routes maintained consistent expression levels until day 11. Intriguingly, IV and IN routes showed no luciferase expression, despite robust *in vitro* expression in Huh7 and A549 cells. Further investigation involved the extraction of primary organs to confirm saRNA presence, and serum collection at 4hrs and 48hrs post-injections to elucidate differences in immunogenic responses when saRNA-LNPs were delivered through different routes. The study now extends to saRNA delivered by pABOL through the same routes.

Conclusion/Significance

This comprehensive study delves into saRNA performance across diverse administration routes using lipid and polymeric systems. The findings provide valuable insights for selecting optimal delivery systems and routes, contributing to the advancement of clinically viable saRNA formulations with substantial commercial value.

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Abstract #26

Development of a high throughput, multi-omic single-cell barcoded *in vivo* LNP screen, to identify mRNA-LNPs with cancer vaccine potential

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Background

Immune activation and antigen presentation are key components of vaccine design. Tumour-antigen/neoantigen-encoded mRNA in lipid nanoparticles (mRNA-LNPs) are of major interest as cancer vaccine candidates. Chemical composition of mRNA-LNPs profoundly impacts immunogenicity, reactogenicity, biodistribution, and subsequent protein production. We developed an *in vivo* high throughput barcoded-mRNA-LNP formulation screening platform harnessing multi-omics, to identify cancer vaccine LNP formulations with desirable antigen-presenting cell targeting and regulatory characteristics. This pipeline, hereafter is referred to as, multi-organ, multi-omic, nanoparticle analysis by sequencing (MOMONA-seq).

Methods

We designed a reporter mRNA (Thy1.1^{SIINFEKL} mRNA) for evaluating LNP formulation immune cell tropism characteristics and antigen presentation potential using antibody-based single cell resolution techniques. Unique barcoded DNA oligonucleotides (BDOs) were encapsulated together with Thy1.1^{SIINFEKL} mRNA in distinct LNPs formulations. The transfection potential of the characterized BDO-mRNA-LNPs was tested in immune cells *in vitro* (flow cytometry and confocal microscopy). The impact of LNP formulation design on blood and tumour immune cell targeting and transcriptional regulation was assessed *in vivo* in tumour-bearing mice using multi-omic scRNA-seq.

Results

Custom designed BDOs were stably co-encapsulated in mRNA-LNPs and, following transfection, were observed in the cytoplasm of dendritic cells. BDO presence did not significantly affect mRNA-LNP physicochemical properties or reporter mRNA translation. MOMONA-seq testing of three FDA-approved LNPs revealed the distinguishing BDOs in immune cells recovered from the processed tissues in their respective hashed samples and in the “pooled” LNP experimental group. ALC-0315-LNP showed the highest BDO reads and protein expression in tumour-associated immune cells, followed by SM-102-LNP and DLin-MC3-LNP, respectively, in both individually administered mice and the “pooled” experimental group. Tumour immune cell subsets differentially uptake LNPs and displayed distinct transcriptional and inflammatory states.

Conclusion

The orthogonal MOMONA-seq workflow successfully allows for high throughput pooled screening of LNPs to identify formulations with preferential cell targeting and regulatory characteristics. The technique allows for simultaneous capture and readout of transcriptional states (mRNA gene expression library), cell-surface protein expression (surface protein library), and LNP barcodes (barcoded LNP library). Immune cell subsets have distinct LNP-mediated mRNA delivery and target cell responses in murine cancer models.

Acknowledgements

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Abstract #27

Novel protamine nanostructures enable sublingual absorption of proteins.

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Background

Therapeutic proteins often required needle-based injections, which compromise medication adherence especially for those with chronic diseases. Sublingual administration provides a simple and non-invasive alternative.

Methods

Herein, two novel self-assembling protamine nanostructures (lipid-conjugated protamine (micelles) and protamine dimer (nano-rod)) were synthesized to enable sublingual delivery of proteins through simple physical mixing with the payloads.

Results

It was found that the protamine nanostructures promoted intracellular delivery of proteins via increased pore formation on the cell surface. Results from *in vitro* models of cell spheroids and human sublingual tissue substitute indicated that the protamine nanostructures enhanced protein penetration through multiple cell layers compared to protamine. The protamine nanostructures were mixed with insulin or semaglutide and sublingually delivered to mice for blood glucose (BG) control.

Conclusions

The effects of these sublingual formulations were comparable as the subcutaneous preparations and superior to protamine. In addition to peptide drugs, the protamine nanostructures were shown to enable sublingual absorption of larger proteins with molecular weights from 22 to 150 kDa in mice, including human recombinant growth hormone (rhGH), bovine serum albumin (BSA) and IgG. The protamine nanostructures given sublingually did not induce any measurable toxicities in mice.

Abstract #28

LNP-R848CuresPeritoneal Metastasis of Colorectal Cancer in Mice

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Background

Colorectal cancer is projected to become the fourth most diagnosed cancer in Canada for 2023. In its late stages, this disease typically metastasizes to the peritoneal cavity. The current therapy is aggressive, and the rate of recurrence is 41% at 1-year post-treatment, 73% at 3 years, and 76% at 5 years [1]. Therefore, there is a pressing need for improved therapies for this indication.

Methods

We have developed a cationic liposomal formulation (LNP) for targeted delivery of TLR7/8 agonist Resiquimod (R848) to the peritoneal cavity. A combination therapy of Oxaliplatin and LNP-R848 were administered intraperitoneally at various dosing regimens and optimized for the best survival (>120 days). Cured mice were then subcutaneously rechallenged with the original cell line. Cure rates using intraperitoneal injection of LNP-R848 and checkpoint immunotherapies was also investigated.

Results

With our optimized dosing regime, we achieved a survival rate of over 120 days of 80%. When rechallenged with the original tumour line, mice were immunized and saw no tumour development. When LNP-R848 was combined with checkpoint immunotherapies, improved survival was observed compared to the individual treatments.

Conclusions

These studies suggest that the usage of our LNP for the delivery of R848 can cure peritoneal metastasis of colorectal cancer in mice and when rechallenged, mice were found to remain tumour free. LNP-R848 was also seen to enhance the anti-cancer effects of immune checkpoint therapies for this indication.

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Abstract #29

A triple adjuvant-based lipid nanoparticle vaccine for influenza: Formulation development, cellular uptake, and cytotoxicity analysis

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Background

A cationic lipid-based carrier for a novel triple adjuvant is being explored for its potential for mucosal vaccination such as intranasal vaccines for influenza. Triple Adjuvant (TriAdj) comprised of poly(I:C), polyphosphazene, and IDR-1002 innate defense regulator peptide was integrated into self-assembled cationic lipid nanoparticles composed of Dimethyldioctadecylammonium bromide (DDAB) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) lipids[1]. This study aims to determine the effect of lipid composition and Lipid:TriAdj ratio on the cellular uptake and cytotoxicity of influenza vaccines in immune cells.

Methods

DDAB/DOPE liposomes (55:45, 50:50, 45:55 mol:mol) were prepared by thin film/extrusion method. L-TriAdj was formulated by admixing pre-formed liposomes with TriAdj at varying ratios. Whole killed H1N1 influenza virus or hemagglutinin (HA) proteins were added to L-TriAdj at a fixed concentration (0.05μg/mL) forming influenza vaccines. Formulations were assessed by dynamic light scattering for particle size and zeta potential (ZP) (Malvern Nano Zetasizer). Cellular uptake of L-TriAdj± H1N1 virus was visualized by fluorescence microscopy (EVOS) using 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE) lipid and quantified by flow cytometry using RAW 264.7 macrophage cells. The mouse dendritic cell line JAWS II was exposed for 24 hrs to L-TriAdj±HA antigen at various lipid:TriAdj ratios and cell viability measured using MTT assays.

Results

L-TriAdj formulations had a desirable size range of 140-180 nm with a consistent surface charge of +40 mV, compared to liposomes alone (100-120nm, ZP>45mV). L-TriAdj uptake in macrophage cells visualized by fluorescence microscopy co-localized the lipid components after uptake into the cytoplasm, with variability in toxicity based on lipid composition and dose. Flow cytometry showed a significant increase in cellular uptake for all L-TriAdj ratios compared to liposomes alone, with >90% of cells positive. Cell viability studies by MTT in cultured dendritic cells clarified optimal lipid:TriAdj ratios.

Conclusion

The L-TriAdj adjuvant system presents a promising path to enhance mucosal vaccine efficacy. Our findings underscore the importance of optimizing DDAB to DOPE ratios and lipid:adjuvant ratio. Significant cellular uptake of the influenza vaccines was noted in macrophages, and cytotoxicity to dendritic cells was minimal, leading towards the identification of lead candidate adjuvants for intranasal influenza vaccine development.

Acknowledgements

This work was supported by a Canadian Institutes of Health Research (CIHR) project grant and the Nanomedicines Innovation Network (NMIN) grant.

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Abstract #30

3-in-1 nanotherapeutic strategies for ovarian cancer

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Background

Among gynecological cancers, ovarian cancer is the leading cause of death in women. Currently, cisplatin is an effective chemotherapy used for treatment. However, chemotherapeutic resistance remains the greatest obstacle in achieving remission. DNA repair and altered metabolism are two resistance mechanisms that prevent effective cytotoxicity. Small molecule drugs, olaparib and metformin, have shown anticancer benefits when combined individually with cisplatin by targeting these two pathways, respectively. Although previous studies have demonstrated that combination therapy has shown stronger efficacy than a single drug alone, non-selective distribution can lead to toxic effects. In this context, cancer nanotechnology has several advantages, including targeted drug delivery. Therefore, I am hypothesizing that the combination of cisplatin, olaparib and metformin encapsulated into a single nanoparticle will improve overall therapeutic effects in ovarian cancer compared to free drug in combination.

Methods

MTT assays were performed in ovarian cancer cells to identify synergistic ratios of drug combinations. To load these ratios inside NPs, cisplatin polymers were synthesized using dicarboxylic acids (linkers) to create repeating cisplatin linker monomers. This modification allows for increased hydrophobicity, thereby increasing encapsulation into polymeric-based nanoparticles. Metformin derivatives with increased hydrocarbon chains were also synthesized to improve hydrophobicity and encapsulation. Self-assembly nanoparticles were synthesized, encapsulating the cisplatin polymers, metformin derivative, and olaparib. Nanoparticles were characterized by size, polydispersity index with Zeta Sizer, encapsulation efficiency with inductively coupled plasma mass spectrometry and high-pressure liquid chromatography, and therapeutic effects.

Results

Synergistic ratios between all the different drug combinations were identified. Results revealed high synergy between specific concentration ranges, guiding nanoparticle synthesis to encapsulate the drugs within these ranges. Cisplatin polymers and metformin derivatives were characterized by proton nuclear magnetic resonance. Percent of platinum in the cisplatin polymers was measured by inductively coupled plasma mass spectrometry revealing roughly 10-14%. Nanoparticle formulations roughly around 120nm are continuously being optimized for a final 3-in-1 particle with ideal parameters. Preliminary results suggest certain formulations of cisplatin polymer nanoparticles have a greater cytotoxic effect than free cisplatin.

Conclusions

Further formulations are required to obtain an ideal 3-in-1 nanoparticle. Once formulated, further *in vitro* and *in vivo* testing will ensue.

Abstract #31

Optimize freeze-drying parameters of lipid nanoparticles to retain mRNA function under long-term storage

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Background

Currently, the storage requirements for mRNA vaccines mandate deep-freeze temperatures, which complicate storage and distribution. This limitation restricts accessibility, particularly in low and middle-income countries. Freeze-drying presents an excellent method to eliminate the need for cold chain transportation and storage of lipid nanoparticle (LNP)-mRNA vaccines by decreasing hydrolysis and mRNA degradation. However, the process of freeze-drying and rehydration often imposes changes to the physicochemical attributes of LNPs and impairs mRNA encapsulation and functionality. Previous study has shown the structure-activity relationship of LNP morphology to mRNA translation as bleb structures improve transfection potency of LNP-mRNA. Thus, in this study we assess the impact of the lyophilization cycle on LNP-mRNA containing the bleb structure.

Methods

First, we formulated and lyophilized LNP-mRNA varying the freeze-drying parameters. We tested out different initial freezing temperatures (-20 °C, -80 °C and with liquid nitrogen at -200 °C), cryoprotectants (sucrose and trehalose) and their concentration (10%, 20% and 30%), formulation buffers (25 mM acetate and 300 mM citrate) and storage buffers (PBS and Tris). Then, we compared the physicochemical characteristics of the reconstituted lyophilized LNP-mRNA to freshly prepared LNP-mRNA by measuring their size, PDI and cryo-EM. Furthermore, we compared the mRNA translatability through transfection efficiency and mRNA encapsulation efficiency. The translatability of reconstituted lyophilized LNP-mRNA was evaluated in mice by *in vivo* imaging studies.

Lastly, we analyzed the colloidal stability of the lyophilized formulation stored at 4°C at various time intervals for long-term storage by measuring the above-mentioned characteristics.

Results

We have proven that with a high enough cryoprotectant concentration, -80 °C is sufficient to preserve morphology of LNP-mRNA. Furthermore, the comparison of different cryoprotectants showed that sucrose was more efficient than trehalose for the lyophilization of LNP-mRNA. Additionally, we examined the cryo-EM and found a correlation between the different morphologies of each formulation and their behavior during freeze-drying, which consequently led to a loss or perseverance of the bleb structure. Lastly, we showed that under those optimized conditions functionality and efficacy of the LNP-mRNA can be maintained as shown *in vitro* and *in vivo*.

Conclusions

Under optimized conditions, we can preserve the bleb structure, showcasing superior performance compared to the Onpattro formulation in both its fresh state and after freeze-drying.

This study provides insight into the lyophilization and long-term storage capabilities of LNP-mRNA containing bleb structures, presenting a potential solution to the global challenge of storage issues.

Abstract #32

Development of miRNA-loaded Targeting Nanoparticles To Alter Cholesterol Efflux In Atherosclerotic Lesional Macrophages

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Background

Current treatments for cardiovascular diseases (CVDs) have primarily focused on lowering low-density lipoprotein cholesterol (LDL-C), yet a significant burden of atherosclerotic disease remains in the absence of high LDL-C due to atherosclerosis-associated inflammation. Consequently, there remains a demand for new therapeutics that can both lower LDL-C and inflammation. Recently, miR-223 (a microRNA or miRNA) has been implicated in modulating cholesterol metabolism based on its ability to regulate cholesterol efflux and inflammation. miR-223 exerts these effects by inhibiting transcription factor Sp3, thus upregulating the ABCA1 transporter on macrophages and inhibiting the NF-κB inflammatory pathways. Despite miR-223's favourable therapeutic outlook, there are limitations in the efficient delivery of miRNA-based therapies *in vivo* due to miRNA's immunogenic nature, rendering it susceptible to degradation. As such, we sought to develop lipid- and polymer-based nanoparticle systems with targeting peptides (S2P and Col IV) to deliver miRNA to macrophages.

Hypothesis:

It is hypothesized that increased miR-223 will inhibit *Sp3* translation, consequently upregulating ABCA1, increasing cholesterol efflux, and lowering NF-κB-induced inflammation.

Methods

We synthesized and characterized several formulations of polymeric- and lipid-based nanoparticles to identify the best formulation for miRNA encapsulation. We assessed their ability to deliver miR-223 to macrophages by monitoring Sp3, and ABCA1 expression. Finally, preliminary assessments in ApoE$^{-/-}$ mice have been conducted to assess NP toxicity and efficacy *in vivo.*

Results

To date, we have optimized several nanoparticle formulations and have identified two polymer- and lipid-based nanoparticles that exhibit desired properties in terms of their size (~82.64 nm), spherical morphology, and miRNA encapsulation efficiency (~80%). Delivery of miR-223 *in vitro* modulates Sp3 and ABCA1 expression. Lastly, preliminary assessments *in vivo* demonstrate capacity of targeted nanoparticles to protect and deliver miRNA to atherosclerotic lesions.

Conclusions

Findings from this study may inform future investigations into the inflammatory effects of miR-223, and the applications of nanoparticles for other diseases.

Abstract #34

Development of a lipid-based triple adjuvant vaccine formulation using a design of experiments (DOE)-based approach

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Background

Conventional subunit vaccines are generally comprised of antigens and adjuvants. Subunit antigens are protein or polysaccharide-based pathogen fragments that generate disease specific immune responses; however, they are poorly immunogenic on their own. Adjuvants are immuno-stimulatory substances added to vaccines to enhance the magnitude, onset and duration of immune responses. The use of a triple adjuvant platform (TriAdj) comprising of three adjuvants has generated potent immune responses for various diseases in prior studies[1]. The co-formulation of TriAdj with cationic lipid nanoparticles generates a nanoparticle complex (L-TriAdj), with utility for intranasal administration, which is of interest for pertussis vaccines. It was hypothesized that the lipid composition plays a significant role in modulating the physicochemical properties of the novel formulation, L-TriAdj.

Methods

In this study, liposomes comprising of varying lipid compositions of dioleoyl dimethylammonium bromide (DDAB), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), cholesterol, and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) or 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were formulated using the thin-film hydration and extrusion method. TriAdj was formulated using simple admixing and added to pre-formed liposomes to form the L-TriAdj complex. Bovine serum albumin, which has similar physicochemical properties to pertactin, a pertussis antigen, was added to L-TriAdj. Design of Experiments (DOE) was performed (DesignExpert™ software) to determine the response surface relationship between the lipid composition and nanoparticle physicochemical properties.

Results

The composition of all four phospholipids had a significant non-linear effect on the particle size and polydispersity (0.2 – 0.4) of the L-TriAdj based vaccine formulation. L-TriAdj nanoparticles containing DSPC had smaller particle diameters (150 – 200 nm) compared to those containing DPPC (200 – 250 nm). The molar ratio of cholesterol was inversely associated with mean diameter and polydispersity index.

Conclusions

The composition of cationic, fusogenic, and structural lipids in L-TriAdj has a significant, interactive, and non-linear effect on the nanoparticles' diameters and variation in diameters. The use of DoE provided a fuller description of the influence of lipid composition on L-TriAdj vaccine physicochemical properties compared to a univariate one-factor-at-a-time approach. The comprehensive results obtained from this study will be used in conjunction with data to be obtained from future immunogenicity studies to determine the structure-activity relationship of L-TriAdj vaccines.

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Abstract #35

LNP based CAR and CRISPR/Cas9 engineering of macrophages for cancer immunotherapy.

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Background

Chimeric Antigen Receptor technology (CAR) has been unsuccessful in treating solid tumours due to the immunosuppressive environment of solid cancers and poor infiltration of lymphoid cells (CAR T and NK cells). Targeting a cell that is found in the tumour microenvironment (TME) could be an effective strategy to overcome this limitation. Macrophages are abundant in the TME and their ability to phagocytose and kill tumour cells make them a highly desirable target for CAR immunotherapy. Macrophage phagocytosis has numerous regulators that can be modulated to enhance cancer cell phagocytosis. We have developed an *in vitro* sgRNA-CRISPR screen to identify genes that inhibit macrophage phagocytosis and that can be targeted to enhance macrophage CAR (M-CAR) mediated cancer cell phagocytosis and killing.

Methods

We utilized mRNA encoding Her2/Neu (Her2)-CAR developed by antibody sequencing to target different cancer models. The transfection potential, antigen binding ability, and the phagocytic ability of the CAR constructs were evaluated in different phagocyte populations *in vitro*. As a proof of concept for the CRISPR screen, a well-characterized phagocytosis regulator, the signal regulatory protein α (Sirpα)/CD47 axis, was targeted. Sirpα was knocked out in murine bone marrow-derived macrophages (BMDMs) as Sirpα on BMDMs binds to CD47 on cancer cells and inhibits phagocytosis.

Results

Multiple anti-Her2/Neu single chain CAR constructs were engineered and tested. The CAR constructs were capable of binding to Her2/Neu protein with high affinity. LNP-engineered CAR-phagocytes showed an enhancement in phagocytosis of different Her2/Neu+ cancer cell types *in vitro*. In the CRISPR screen, SIRPα was efficiently knocked out in targeted BMDMs by LNP delivery of sgRNA and greatly improved anti-Her2/Neu dependent phagocytosis of cancer cells *in vitro.*

Conclusions

We demonstrated the anti-cancer potential of CAR macrophages *in vitro.* Additionally, we developed an *in vitro* sgRNA-CRISPR screen to identify negative regulators of phagocytosis and potentially other physiological processes useful against cancer. Future work will be aimed at validating our findings *in vivo* in preclinical cancer models and optimizing LNP delivery systems allowing for combined M-CAR production and macrophage genetic modification to improve cancer immunotherapy.

Abstract #36

Lipid nanoparticles formulated in the presence of 300 mM sodium citrate enable enhanced *in vivo* gene editing with CRISPR/Cas9 adenine base editor mRNA and sgRNA

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Background

Achieving efficient and safe delivery of gene editing systems is a critical step in enabling future therapeutics for the treatment of genetic disorders. As opposed to viral vectors, lipid nanoparticles (LNPs) containing RNA present an especially appealing delivery method due to having lower immunogenicity, readily scalable manufacturing processes, and transient expression of gene editing components that lower the chances of unwanted off-target editing. Work from the lab of Dr. Pieter Cullis recently demonstrated that using 300 mM Na-citrate as the pH 4 loading buffer during LNP synthesis greatly improved functional delivery of mRNA, likely due to improved mRNA stability [1]. We reasoned that incorporating this into the LNP formulation process for base editor mRNA and sgRNA could, therefore, also improve gene editing.

Methods

As a model to assess gene editing, we utilized our LumA luciferase reporter mouse [2], where successful gene editing of a defective luciferase transgene results in restoration of luminescent signal, allowing visualization of the efficiency and biodistribution of gene editing. Mice were given a single dose of KC2 ionizable lipid LNPs containing adenine base editor mRNA and sgRNA, formulated using either standard 25 mM sodium acetate or 300 mM sodium citrate as the pH 4 buffer. Gene editing was assessed using IVIS whole-body live imaging, IVIS imaging of extracted organs, and terminal tissue luciferase assays.

Results

We found that the use of the 300 mM Na-citrate pH 4 buffer resulted in strikingly higher gene editing efficiency. Live imaging and terminal luciferase assays indicated a ~7x and ~10x increase in gene editing with the 300 mM Na-citrate formulation relative to LNPs prepared using the 25 mM Na-acetate pH 4 buffer.

Conclusions

This work indicates that the use of the 300 mM Na-citrate buffer in LNP formulation is a promising approach to enhance the efficiency of RNA-based LNP-mediated gene editing. In the future, we aim to investigate the use of this buffer modification in LNP-mediated gene editing applications using ionizable lipids other than KC2 and in delivery to extrahepatic tissues.

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Abstract #37

A magnetic separation method for isolating and characterizing the biomolecular corona of lipid nanoparticles

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Background

Lipid nanoparticle (LNP) formulations are a proven method for the delivery of nucleic acids for gene therapy. Enroute to their target, LNPs interact with biological fluids (i.e. blood), components of which adsorb onto the LNP surface forming a layer called the "biomolecular corona" which affects LNP stability, biodistribution, and tissue targeting. Technical hurdles in corona isolation combined with the fact that corona composition changes in different species and patients have made LNP-cell interactions difficult to predict, and correlation between *in vitro* and *in vivo* models is almost absent. Therefore, this project aims to understand and exploit the corona of clinically relevant gene therapeutics to improve their clinical applications.

Methods

In this study, LNPs were formulated with a superparamagnetic iron oxide core and incubated with human serum. This allowed the separation of LNP-corona complexes from unbound serum proteins using magnetic separation. In parallel, size exclusion chromatography, a common technique used to study the corona, was performed. The corona composition of each separation method was analyzed by mass spectrometry.

Results

Over 200 proteins were identified for the LNP-corona samples separated via magnetic separation and size exclusion chromatography. Although common serum proteins including ApoE and albumin were present in LNP-corona complexes, significant differences in protein abundance between the two separation methods were observed.

Conclusions

This study illustrates the importance of using more effective and reliable separation strategies of an intact LNP against unbound proteins. This can reveal new structural information and insight toward LNP-corona complexes.

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