Lipid nanoparticles for organ-specific delivery of RNA

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ABSTRACT SUMMARY

Novel nanoparticulate formulations for organ-specific delivery of nucleotides were developed. After intravenous (i.v.) injection into mice, RNA could be targeted either to lung or spleen, depending on the composition of the nanoparticles.

For targeting selectivity, certain physicochemical parameters of the lipoplex nanoparticles, together with different uptake mechanisms in the target cells were considered to be decisive. Thorough physicochemical characterization enabled us to obtain insight into the structure-function correlations, and conditions for the assembly of stable formulations, appropriate for pharmaceutical use were defined.

The novel formulations are to be used in tumor immunotherapy to deliver RNA to the spleen. First clinical trials are in preparation.

INTRODUCTION

Messenger RNA (mRNA)-based nanomedicines constitute a new class of pharmaceutical products, with a variety of potential applications, ranging from tumor immunotherapy to protein substitution. In tumor immunotherapy, tumor antigen-encoding mRNA is to be delivered into APCs in order to induce T-cell mediated antitumoral responses. One key prerequisite for successful translation of this concept into clinical practice is efficient delivery of the mRNA to the target cells, including subsequent cellular uptake and expression of the antigen. So far approaches for systemic administration of mRNA medicines have been hampered by poor serum stability and/or insufficient targeting efficacy and selectivity of the compounds.

Here, we report on RNA lipid nanoparticle formulations that, after i.v. administration, specifically target selected organs, i.e. lung or spleen. Targeting selectivity was obtained by selecting the lipid and RNA composition, the ionic conditions for particle formation and on other physicochemical parameters like size or lamellarity. Based on a composition for spleen targeting, novel mRNA nanomedicines for tumor immunotherapy were developed. Components and assembly strategies were selected in a way to enable straightforward upscaling and GMP manufacturing of clinical trial batches. On this basis, for the first time, a formulation for efficient and highly selective delivery of RNA to the spleen was translated into clinical development. Several clinical trials are underway or in preparation.

EXPERIMENTAL METHODS

Lipids (DOTAP, DOTMA, DOPE, DOPC) were obtained from Avanti Polar Lipids Inc. (Alabama, USA), luciferase coding RNA was synthetized by internal protocols. NaCl, Ethanol 99.5% Ph.Eur. was from Carl Roth (Karlsruhe, Germany), PBS was from Life Technologies GmbH (Darmstadt, Germany) and RNase free water, Aqua, from B. Braun Melsungen AG (Germany). Liposomes were extruded using a Lipex™ 10 mL extruder (Northern Lipids, Burnaby, Canada) and polycarbonate membranes with different pore sizes. The lipid concentration in the liposome dispersions was controlled by HPLC and the RNA concentration was determined by UV spectroscopy. RNA lipid nanoparticles (lipoplexes) were formed by incubation of the RNA with ions, buffers, and liposomes using different relative concentrations of the respective components and mixing protocols. Particle size and zeta potential were determined by dynamic light scattering using a 380 ZLS particle analyzer (PSS Nicomp, Santa Barbara, CA). Synchrotron small-angle X-ray scattering (SAXS) experiments were performed at the Soft Condensed Matter beamline A2, HASYLAB, DESY (Hamburg, Germany) using a monochromatic radiation of 0.15 nm wavelength. Uptake and translation of formulated firefly luciferase-encoding RNA (luc RNA lipoplexes) were evaluated by in vivo bioluminescence imaging using the Xenogen IVIS Spectrum imaging system (Caliper Life Sciences).

RESULTS AND DISCUSSION

For the formulations described here, different compositions of cationic lipids, so-called helper lipids, ionic (buffer) conditions, particle characteristics and preparation protocols were varied, but no specific ligands were used.

In Fig. 1 the results of particle size and zeta potential measurements for different lipid/RNA compositions are shown. Liposomes consisting of a cationic lipid and a helper lipid were mixed with RNA in different ratios. With an excess of either negatively or positively charged...
moieties, stable colloidal dispersions were obtained. In this range, the zeta potential of the particles was constant and either negative or positive. For all tested lipid compositions the colloidal stability was assessed in the described way. Such well-defined preparations were selected for further biological evaluation.

In Fig. 1, results from small angle x-ray scattering (SAXS) measurements from selected RNA lipid nanoparticle formulations are shown. In all cases, a clear Bragg diffraction peak, indicative for an organized structure with defined repetitive spacing was determined. In contrary, no Bragg peaks were found for the pure liposomes (data not shown).

The d-spacing, calculated as \( d = 2\pi q_{\text{max}} \) was in the order of 6 to 6.5 nm and depended on the composition and ionic conditions. In the presence of buffers, higher spacings (peak maxima at lower \( q \)) were observed. Assuming an organization, were RNA is sandwiched in between lipid bilayers, this would point towards a higher amount of (compacted) RNA inserted in between the layers in the presence of buffer. Formulations from unextruded liposomes had the same d-spacing as the extruded ones however the peak width was lower, indicating a higher correlation length (higher number of layers in the stack). In vivo, formulations formed in the presence of ionic buffers and from unextruded liposomes showed higher activity in vivo compared to formulations, where this was not the case.

In Fig. 3, results from in vivo imaging of luciferase expression of two different formulations, selected from the compositions as shown in fig.1 are shown. Formulation A was with excess negative charge (+/-1/2) and B was with excess positive charge (+/- 4/1). In both cases, pronounced luciferase expression was observed, indicating that the RNA was protected from degradation and delivered to the organs. In one case (A), preferential lung targeting was observed, while with in the other case (B), high targeting selectivity for the spleen was detected. Further measurements on the cellular level (e.g. FACS) indicated that in the spleen, the splenocytes were the preferential target of the RNA nanoparticles.

CONCLUSION
Novel RNA nanomedicines for i.v. administration were developed that enabled efficient and organ-selective expression of the RNA. The biological activity and selectivity could be adjusted by altering physicochemical parameters of the formulations. Based on this technology, several new RNA nanomedicine products could be translated into clinical development.

REFERENCES

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Fig. 1: Size and zeta potential of RNA lipid nanoparticles as a function of the molar ration between cationic lipid (here using DOTMA with DOPE as co-lipid at molar ratio 1/1) and RNA (nucleotides).

Fig. 2: SAXS measurements from RNA lipid nanoparticles (F1: DOTMA/DOPE/RNA 2/1/1; F2: 2/2/1) in water and in PBS buffer. Only the range of the Bragg peaks is shown. Curves were vertically shifted for clarity.

Fig. 3: Luciferase expression in vivo (left) and ex vivo in the organs (right) 6h after injection of 20 µg of RNA; formulation A charge ratio: 4/1, B: 1/2