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RESEARCH ARTICLE

Toxicity profiling of several common RNAi-based nanomedicines: a comparative study

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Abstract RNAi-based nanomedicine platforms (RNPs) have progressed from tools to study gene expression in vitro into clinical trials. Numerous RNPs strategies have been documented with an efficient ability to condense RNAi payloads and induce potent gene silencing. Moreover, some of these RNPs have been explored in various animal models, and some have even made it to the clinic. Still, there is lack of a clinically approved RNAi-based delivery strategy most probably due to unpredicted clinical toxicity. In this study, we prepared common RNPs such as cationic liposomes, polyamines, and hyaluronan-coated lipid-based nanoparticles and tested these strategies for global toxicity parameters such as changes in bodyweight, liver enzyme release, and hematological profiling. We found that polyamines such as polyethyleneimine and Poly-L-lysine released high levels of liver enzymes into the serum and reduced C57BL/6 mice bodyweight upon three intravenous injections. In addition, these polyamines dramatically reduced the total number of leukocytes, suggesting an immune suppression mechanism, while cationic liposomes, which also increased liver enzymes levels in the serum, elevated the total number of leukocytes probably by activation of Toll-like receptors 2 and 4. Coating the liposomes with hyaluronan, a hydrophilic glycosaminoglycan, provided a protective layer and did not induce adverse effects upon multiple intravenous injections. These findings suggest that there is an urgent need to develop gold standards for nanotoxicity in the field of RNAi that will be embraced by the RNAi community.

Keywords RNAi · Nanomedicines · Nanotoxicity · Cationic liposomes · Polyamines

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Introduction

RNAi is a specific regulatory mechanism of most eukaryotic cells, in which small double-stranded ribonucleic acids efficiently control gene expression in a complementarity-dependent manner. This natural mechanism, which involves diverse families of small non-coding RNA regulators, is presumed to protect against pathogenic infections as well as regulate various biological pathways [1, 2]. Utilizing this native mechanism for therapeutic and diagnostic purposes can be achieved by exogenous delivery of synthetic RNAi molecules. However, due to their net negative charge and relatively large size compared with vasculature effective pore size, RNAi molecules are less likely to readily cross biological barriers, thus, efficient systemic delivery necessitates the use of physical protection (i.e., carriers/reagents) and chemical modifications that together protect RNAi molecules until reaching their site of action and facilitate cell penetration, yet maintaining their functionality [2, 3]. Numerous strategies to deliver RNAi have been developed with some promising in vivo results [4, 5].

Despite the promise, developing RNAi as therapeutics has proven challenging. Like most drug development, there is quick fix, and many pharmaceutical companies that invested considerable sums in developing RNA-based drugs announced they are closing their RNAi subsidiaries.

Although many of the hurdles in developing RNAi-based therapeutics have been already addressed, the main challenge is figuring out the way of delivering small RNAs into cells in a therapeutically relevant manner with minimal adverse effects. Several delivery strategies for RNAi payloads have been well documented over the past years, among them polyamines such as poly-L-lysine and polyethyleneimine (PEI) [6–8], cationic liposomes [9–11], and neutral lipid-based nanoparticles [12–15]. In these delivery strategies, the efficiency of gene silencing and phenotypic genes have been documented, yet comprehensive analyses of adverse effects were not reported for all

these strategies. Herein, we profiled global toxicity of four representative delivery strategies, poly-L-lysine, PEI, cationic liposomes, and neutral lipid-based nanoparticles all entrapping or condensing siRNA payloads with an effective knockdown capabilities. We examined global changes in bodyweight upon three intravenous administrations of each of the delivery systems with its RNAi payloads, tested liver enzyme release in the serum of mice, and profiled total leukocytes and specific subsets count upon multiple intravenous (IV) injections into mice.

Materials and methods

Materials Linear PEI (750 kDa), poly-L-lysine, were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP) was obtained by Avanti Polar Lipid (Albaster, AL, USA). Other lipids were from Avanti Polar Lipids Inc. (Alabaster, AL, USA) or Phospholipid GMBH (Germany). Sodium hyaluronate (HA) with an average molecular weight of 700 KDa was obtained from Lifecore Biomedical Co. (Chaska, MN). All other reagents were from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of lipid-based nanoparticles (LNPs)

Multilamellar vesicles (MLV) composed of pure soybean phosphatidylcholine (Phospholipon 90G), which was a kind gift from Phospholipid GMBH (Germany). 1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) and cholesterol (Chol) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). PC/Chol/DPPE were at mole ratios of 60:20:19.9, with the addition of 0.1 % DPPE labeled with rhodamine fluorophore (Invitrogen) were prepared by the traditional lipid-film method [16–19]. Briefly, the lipids were dissolved in ethanol and evaporated to dryness under reduced pressure in a rotary evaporator (Buchi Rotary Evaporator Vacuum System Flawil, Switzerland). Following evaporation, the dry lipid film was hydrated in 10 ml of HEPES (pH 7.4). This was followed by extensive agitation using a vortex device and 2 h incubation in a shaker bath at 65 °C. The MLV were extruded through a Lipex extrusion device (Northern lipids, Vancouver, Canada), operated at 65 °C and under nitrogen pressures of 200–500 psi. The extrusion was carried out in stages using progressively smaller pore-size polycarbonate membranes (Whatman Inc, UK), with several cycles per pore-size, for achieving unilamellar vesicles in a final size range of ~100 nm in diameter. Lipid mass was quantified as previously reported [19, 20].

Hyaluronan-coated–LNPs

We have previously shown that high-molecular weight hyaluronan (HA), of an average of 700 kDa from Lifecore Biomedical LLC (Chaska, MN, U.S.A) when coated onto the surface of LNPs, endow these particles with high affinity to CD44, its natural receptor [19]. The HA was dissolved in 0.2 M MES buffer (pH 5.5) to a final concentration of 5 mg/ml. HA was activated with EDC and sulfo-NHS at a molar ratio of 1:1:6. After 30 min of activation, the LNPs were added, and the pH was adjusted to 7.4. The solution was incubated at room temp (2 h). The free HA was removed by 3 cycles of repeated washing by centrifugation ($1.3\times10^5\times g$, 4 °C, 60 min). HA was quantified as previously demonstrated [19, 20]. The final HA/lipid ratio was typically 75 μg HA/μmole lipid as assayed by ^{3}H-HA (ARC, Saint Louis, MI) with an average of 2 % of the particles that had the HA decoration.

Particle size distribution and zeta potential measurements

Particle size distribution and mean diameter of the HA-coated lipid-based nanoparticles (HA-LNPs), PEI-siRNA, and PLL–siRNA complexes as well as cationic liposomes and control, non-surface-modified NPs (LNPs) were measured on a Malvern Zetasizer Nano ZS zeta potential and dynamic light scattering instrument (Malvern Instruments, Southborough, MA) using the automatic algorithm mode and analyzed with the PCS 1.32a. All measurements were done in 0.01 mol/l NaCl, pH 6.7, at 20 °C.

siRNA encapsulation or complex formation.

siRNA sequences against the CCND1 gene NM_053056 (siD1, sense strand: GUAGGACUCUCAUUCGGGATT) and the luciferase gene as a control sequence (siLuc, sense strand: CUUACGCUGAGUACUUCGA) were designed and screened by Alnylam Pharmaceuticals (Cambridge MA, USA) and previously published [21]. HA-LNPs siRNAs were entrapped in HA-LNPs or LNPs as previously reported [15]. Briefly, HA-LNPs and LNPs were lyophilized until complete water removal was ensured (48 h). The lyophilized particles were hydrated with DEPC-treated water with or without siRNAs. The particles were shaken gently for 30 min at room temperature. Unencapsulated siRNA was removed via ultra-fast centrifugation at $6.4\times10^5\times g$, 4 °C, 20 min prior to adding the suspension to the cells or to intravenous injection into C57BL/6 mice.

PEI–siRNA complex formation To formulate the siRNA–PEI complex, 200 μ l of siRNA (0.1 mg/ml) was condensed with 100 μ l of PEI (0.6 mM) in 10 mM HEPES buffer (pH 7.4), at a nitrogen/phosphate ratio of 1.0.

Poly-L-lysin (PLL)–siRNA complex formation To formulate the siRNA–PLL complex, 200 μl of siRNA (0.1 mg/ml) was condensed with 200 μl of PLL (10KDa , 0.3 mM) in 10 mM HEPES buffer (pH 7.4).

Preparation of Cationic (+) LNPs and siRNA entrapment (+)LNPs were composed of HSPC/Chol/DOTAP at 3:1:1 mole ratio and prepared as detailed above for LNPs.

siRNAs entrapment was done similar to the one reported in Kedmi et al. [22]. Briefly, the lipids were dissolved in chloroform/methanol (4:1, *v/v*). The organic solvent was evaporated under pressure at 60°C for 30 min, and the lipid film was flushed with N_2 gas to remove residual solvent. The lipid film was hydrated using a solution of siRNAs (Luciferase , as control or CyD1) with previously described sequence [21] in 5 % dextrose (*w/v*) prepared using RNase-free dH_2O. Size reduction was performed as with conventional particles (see above).

siRNA quantification of the efficiency of entrapment

siRNA encapsulation efficiency was determined by the Quant-iT RiboGreen RNA assay (Invitrogen) as previously described by us and others [13, 15, 20, 23]. Briefly, the entrapment efficiency was determined by comparing fluorescence of the RNA binding dye RiboGreen in the (+)LNP, HA-LNP, PLL-siRNA, and PEI-siRNA complexes samples, in the presence and absence of 0.5 % Triton X-100 [13, 15]. In the absence of detergent, fluorescence can be measured from accessible (un-entrapped or condensed) siRNA only. Whereas, in the presence of the detergent, fluorescence is measured from total siRNA [23]; thus, the % encapsulation is described by the equation:

$$\%\ \text{siRNA encapsulation/complexation} = [1-(\text{free siRNA conc.}/\text{total siRNA conc.})] \times 100.$$

Based on the entrapment efficiency, we have calculated the specific amount needed for each formulation for in vitro and in vivo experiments so no bias will occur in the results or while interpretation of the data.

Delivery of siRNAs into cancer cells by RNPs

Human lung adenocarcinoma cell line (A549) were seeded into six-well cell culture plates at 0.1×10^6 cells/well in RPMI medium, supplemented with antibiotics, L-glutamine, and 10 % fetal calf serum (Biological industries, Beit Haemek, Israel). Twenty-four hours post-seeding the medium was removed and replaced with RPMI only. The cells were then treated with empty vehicles (HA-LNPs) or with tested RNAi-based nanomedicine platform (RNP) (HA-LNPs, PLL, PEI, or (+)LNPs) encapsulating Cyclin D1 (CyD1) or luciferase (Luci) siRNAs. CyD1 chosen as a surrogated marker to evaluate the delivery strategy. Four hours post-incubation, the medium was removed, and the cells were washed and supplemented with complete medium. Three days after transfection, the cells were split 0.2×10^6 cell/well into fresh 6 wells cell culture plate. Final siRNA concentration on the cells was 100 nM.

Quantitative real-time PCR

QPCR was done as previously published [21]. Briefly, total RNA was isolated using EZ-RNA kit (biological industries, Israel), and cDNA was generated with high-capacity cDNA kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturers' protocols. qRT-PCR was performed with Fast SYBR® Green Master Mix and the ABI StepOnePlus™ instrument (Life Technologies). CCND1 (F:GAGGAGC CCCAACAACTTCC, R:GTCCGGGTCACACTTGATCAC) expression was normalized to the house keeping genes eIF3a (F:TCCAGAGAGCCAGTCCATGC, R:CCTGCCACAATT CA TGCT) and eIF3c (F:ACCAAGAGAGTTGTCC GCAGTG, R:TCATGGCATTACG GATGGTCC). Analysis was done with the StepOne™ software V 2.1 (Life Technologies) using the multiple endogenous controls option. When using multiple endogenous controls, the software treats all endogenous controls as a single population and calculates the experiment-appropriate mean to establish a single value against which the target of interest is normalized.

Animal care and treatment

Animals (healthy C57BL/6 mice) were obtained from the animal-breeding center, Tel-Aviv University (Tel Aviv, Israel). Animals were maintained and treated according to National Institutes of Health guidelines. All animal protocols were approved by the Tel-Aviv Institutional Animal Care and Use Committee.

Bodyweight measurements

Healthy C57BL/6 mice (n=7/group) were given either a single or three bolus IV injections of saline or treated with (+)LNPs-siRNA, PLL-siRNA, PEI-siRNA, and HA-LNP (with siRNAs) at days 4, 8, and 12 from experiment initiation at a dose of 1 mg/kg body siRNA/dose/mouse), and individual mouse bodyweight was monitored every 4 days using an Avery Berkel scale (Fairmont, MN, USA). Cyclin D1 –siRNA was used in all tested groups.

Blood biochemistry and hematology assays

Healthy C57BL/6 mice (n=7/group) were given three bolus IV injections of saline (+)LNPs-siRNA, PLL-siRNA, PEI-

siRNA, and HA-LNP (with siRNAs) at days 0, 2, and 4 from experiment initiation at a dose of 1 mg/kg body siRNA/dose/mouse); 24 h after the last injection, blood was drawn, and the serum was obtained by centrifugation of the whole blood at 850×*g* for 15 min in a protocol we previously reported [22]. Liver enzyme levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and cholesterol levels were determined by COBAS MIRA auto analyzer (Roche). In addition, the number of major groups of leukocytes was also measured.

Statistical analysis In vitro data were analyzed using Student's *t*test. Differences between treatment groups were evaluated by one-way ANOVA with significance determined by Bonferroni-adjusted *t*tests.

Results and discussion

Structural and physicochemical characterization of RNAi-based nanomedicine platforms

In order to compare different toxicity parameters of RNPs in vivo, we prepared four different RNPs and complexed or entrapped siRNAs (all with same siRNA concentration, 100 nM for in vitro use and 1 mg/kg body siRNAs for in vivo application). Size distribution, zeta potential, and efficiency of RNAi payload entrapment or complexation of PEI-, PLL-based siRNA complexes, (+)LNP-siRNA, and HA-LNPs entrapping siRNA are detailed in Table 1.

All RNPs-siRNA had mean diameter of <200 nm with narrow distribution pattern. PEI, PLL, and (+) LNPs had a positive zeta potential that was decreased with the addition of siRNAs (data not shown), while HA-coated LNPs had negatively zeta potential.

Table 1 Physicochemical and structural characterization of RNAi-based nanomedicine platforms

RNAi-based nanomedicine platform	Size distribution (nm)	Zeta potential (mV)	siRNA entrapment/ complexation efficacy (%)
PEI-siRNA	155.5±7.4	15.3±2.1	88.3±4.6
PLL-siRNA	162.2±9.4	9.7±1.8	62.8±7.2
HA-LNPs–siRNA	130.9±11.2	−25.4±2.9	60.4±2.2
(+)LNPs–siRNA	189.5±8.8	42.4±2.7	90.3 ±2.7

Each result is an average (±SD) of six independent measurements. Particles were measured at pH 6.7, in ddH_2O with 10 mM NaCl, at 20° C using a Malvern ZS Zetasizer

Robust knockdown of cyclin D1 in cancer cells by RNPs

We compared all four strategies to assess the knockdown efficiency in a model cancer cell, a human lung adenocarcinoma cell line (A549). These cells also highly expressing the HA receptor, CD44, as we recently showed [15]. The experimental setting is detailed in the "Materials and methods" section, and we chose CyD1 as a surrogated marker since A549 highly express CyD1 [24]. Around 65 % knockdown of CyD1 mRNA levels was observed 20 h post-transfection with all four delivery systems (Fig. 1a) with no significant differences between the four strategies. This knockdown was specific for all four types of delivery strategies as observed from a control study with Luci-siRNA (Fig. 1b).

Decrease in bodyweight is observed upon intravenous injections of cationic formulations

Next, we examined global changes in bodyweight in healthy mice injected with all four delivery systems upon single and multiple [3] intravenous injections (Fig. 2).

We chose a therapeutically relevant dose of 1 mg/kg body siRNA that was shown effective in vivo in all formulations [22, 25–27]. A single IV injection 4 days post-initiation of the experiment shows that, in all cationic formulations (PLL-siRNAs, PEI-siRNAs and (+)LNPs-siRNAs), a slight reduction in bodyweight was observed to a certain degree; however, due to large standard deviations, it is not significantly different than the mock-treated group (Fig. 2a). Screening the results for multiple IV injections (once every 4 days) reduced the bodyweight of all formulations except that of the HA-coated LNPs and the mock-treated groups (Fig. 2b). No reduction in bodyweight was observed when siRNAs were applied in saline with 5 % glucose (data not shown), implying that the siRNAs itself is not the cause for this general toxicity in the cationic formulations.

Positively charged RNPs induce liver enzyme release in the serum of healthy mice

Potential systemic toxicity can be assayed by global toxicity markers [20, 28] such as serum liver enzymes and body weight loss upon multiple injections. Since we have already witnessed bodyweight loss upon multiple IV injections (in the case of the cationic RNPs), we wanted to examine the levels of liver enzyme release (as probes for short-term hepatotoxicity and cholesterol levels as long-term hepatotoxicity) [22].

Serum levels of the liver enzymes, ALT and AST (Fig. 3a), and cholesterol levels (Fig. 3b) were determined in healthy C57BL/6 mice (n=7/group) administrated

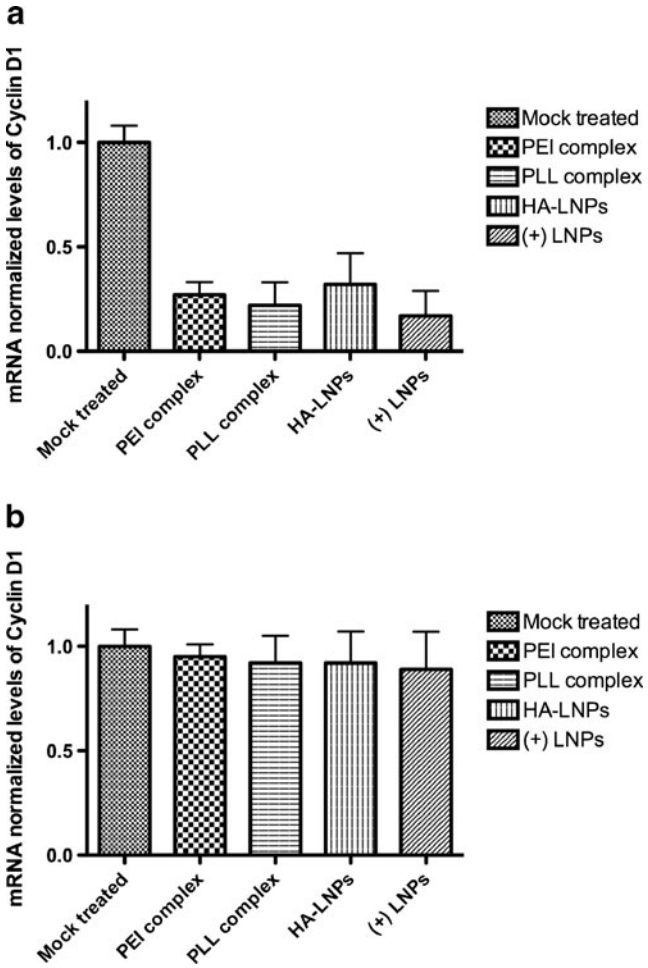

Fig. 1 Robust knockdown of CyD1 in cancer cells by RNPs. mRNA levels of CyD1 upon transfection of A549 cells with different RNPs quantify using QPCR 24 h post-transfection. **a** CyD1-siRNAs were formulated in all four different delivery strategies. **b** A control study with luciferase–siRNA formulated in all the four different RNPs, demonstrating the specificity of the CyD1 sequence. Data are average of four different experiments, and the *error bars* represent the SD values between these independent experiments

intravenously with saline or with 1 mg/kg body of PLL-siRNA, PEI-siRNA, (+)LNPs, and HA-coated LNPs, and 24 h after the last IV injection, blood was drawn to assess potential hepatotoxicity.

Examining liver enzyme release into the serum after 24 h post-injection is considered to be a gold standard for examining acute hepatotoxicity [13, 22]; therefore, we chose to draw blood at this time point.

Administration of (+)NPs–, PLL–, and PEI–siRNA significantly enhanced liver enzymes release 24 h after the last injection (six- to tenfold higher than the mock-treated mice), indicating possible short-term liver toxicity. However, serum cholesterol levels remain the same in all tested RNPs, indicating that the potential toxicity is transient, and long-term cholesterol synthesis was not affected by these short-term hepatotoxicity. Administration of naked siRNA did not trigger any release of liver enzymes or changed cholesterol levels (data not shown), demonstrating that these modified molecules are safe for use.

Cationic NPs may cause immune suppression or immune activation

We next examined the total number of leukocytes upon three IV administrations into C57BL/6 mice. HA-coated LNPs had comparable numbers of leukocytes to the mock-treated group (Table 2), suggesting a protective mechanism of the HA layer. PLL- and PEI-based platforms showed dramatic decrease in the total number of leukocytes as well as in hemoglobin levels (Table 2) with no change in the distribution pattern between lymphocytes and monocytes—this might be explained by general cell death via damage to the mitochondria and other cellular organelles. In addition, a recent study, demonstrated that,

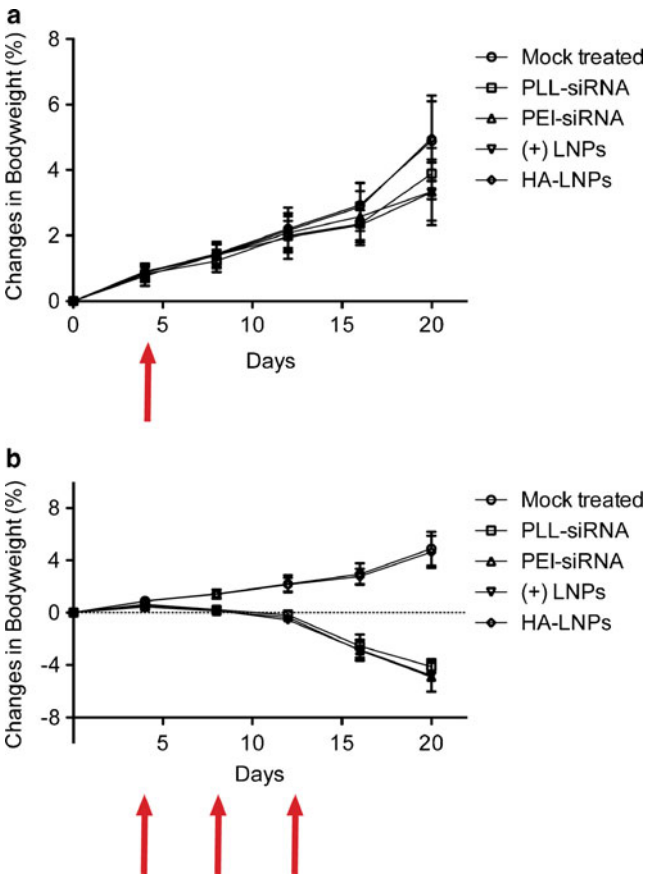

Fig. 2 Changes in bodyweight upon intravenous injections of different RNPs-siRNAs is observed in cationic formulations. Bodyweight changes (%) was monitored over a period of 16 days after the first IV administration. **a** Single IV administration. **b** multiple [3] IV administrations. *Arrows* (*red*) represent the days of IV administration. Reduction in bodyweight was observed in all the cationic formulations. Data presented is an average±SEM of $n=7$ mice/group for each experiment

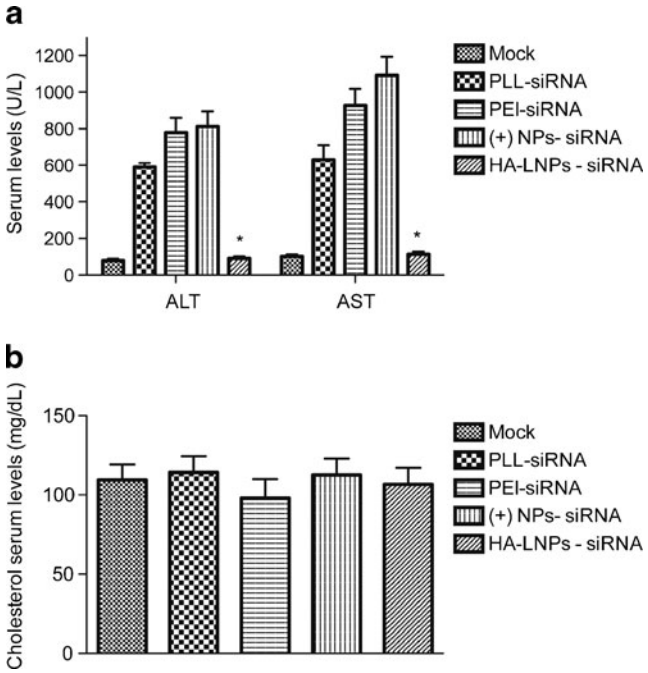

Fig. 3 Positively charged RNPs induce liver enzyme release in the serum of healthy mice. **a** Liver enzyme release profile, which is often regarded as probe for transient liver toxicity was assayed upon three IV injections of various RNPs into healthy C57BL/6 mice (n=7/group). All the cationic RNPs elevated both ALT and AST, six to tenfold compared with mock-treated group) 24 h after the last injection. **b** Serum cholesterol levels, which are considered as probes for long-term liver toxicity did not show any changes between the groups, which could indicate that the damage to the liver is transient. Data presented is an average±SEM of n=7 mice/group for each experiment. *Asterisk* indicates $p<0.001$

besides necrosis and apoptosis, autophagy is associated with PEI- and PLL-induced cytotoxicity and contributed to aggravated cell damage [29].

Increased in the total number of leukocytes as well as the percentage of lymphocytes and monocytes was observed when (+)LNPs were administrated multiple times. This observation might be explained by a cascade of immune activation which causes proliferation of leukocytes. We have previously shown that (+)LNPs trigger both cytokine induction and interferon release in a Toll-like receptor (TLR)4-specific manner. This immune activation leads also to leukocyte proliferation [30–32].

Immune activation often initiates when nanoparticles interact with cells of the innate immune arm such as monocytes, macrophages, and dendritic cells , in a similar manner to a pathogen infection or being sensed as a damaged tissue. This interaction may lead to signal cascades upon activation of pattern recognition receptors (PRRs). PRRs are proteins expressed by cells of the innate immune arm to identify pathogen-associated molecular patterns, which are associated with microbial pathogens or cellular stress [30, 33].

It is clear from our data that coating LNPs with HA endows a protective layer from immune activation.

The use of HA on LNPs as well as on other particles surface such as PEI or PLL may endow these carriers with long-circulating and potentially tumor-targeting capabilities to HA receptors, CD44, and CD168, which are highly expressed on tumor cells, as we and others previously demonstrated for other HA-coated nanovectors [17, 18, 34, 35].

Conclusions

In this study, we have compared four established RNPs and assessed their general toxicity in vivo. We found that all four strategies tested namely polymeric polyamines such as PLL and PEI, cationic lipid-based nanoparticles ((+)LNPs), and hyaluronan-coated LNPs (HA-LNPs) entrapping or complexing siRNAs induced robust gene silencing in vitro in an established human lung adenocarcinoma cell line

Table 2 Hematological profiling of RNPs upon multiple injections into C57BL/6 mice

	Normal ranges	Mock-treated	PLL	PEI	(+)LNPs	HA-LNPs
PCV (%)	39–44	44±2	42±1	41±4	41±3	43±2
Hgb (g/dl)	10.2–16.6	13.4±2.1	9.4±0.4*	8.9±0.5*	14.7±3.4	13.9±1.5
WBC (×1,000)	6–15	7.5±1.5	4.4±1.0*	4.0±0.4*	20.2±1.2*	9±1.0
Diff. (%)						
Lymphocytes	55–65	60±4.5	53±5	59±9	77±1.3*	59±5.2
Monocytes	1–4	3±0.5	2±1.5	3±1	8±1.1*	4±0.3
Eosinophils	0–4	2±0.1	1±0.4	2±0.6	0	3±0.1
Basosphils	0–1	0	0	0.5±0.1	0	0

Results are an average ±SEM of n=7 mice/tested group. All RNPs had siRNAs against CyD1 at 1 mg/kg body

PCV packed cell volume which is a measure of the proportion of blood volume that is occupied by red blood cells, *Hgb* hemoglobin levels, *WBC* (×1,000/uL) white blood cell counts, *Diff. (%)* percent of differentiated leukocytes

*$p<0.001$ compared with the mock-treated group

A549. However, when injected systemically, three intravenous injections separated by 4 days apart, all the cationic formulations decreased the bodyweight of C57BL/6 mice. Moreover, the cationic formulations induced liver enzyme release, indicating a potential transient hepatotoxicity. No changes have been observed with cholesterol levels in all injected RNPs, suggesting that no long-term toxicity is happening with these low concentrations of siRNAs (1 mg/kg body). Interestingly, both polymeric polyamines decreased the total number of leukocytes and hemoglobin, suggesting global cellular toxicity and perhaps immune suppression mechanism, while cationic LNPs dramatically increase the total number of leukocytes suggesting immune activation, most likely in a TLR4-dependendt manner. HA-coated LNPs did not alter the number of leukocytes nor changed bodyweight or trigger the release of liver enzyme suggesting a protective layer of the HA coating. Previously, the group of Leaf Huang has shown that coating LNPs with high-molecular-weight HA endows these carriers with minimal to no toxicity when injected systemically into mice bearing tumors. Our group has shown that no immune toxicity is occurring when HA is used as a scaffold for binding targeting agents to the surface of LNPs when modulating leukocytes function during inflammatory bowel diseases and in targeting T helper cells for suppressing HIV infection [20, 27, 34, 36, 37].

Taken together, the results presented here support the use of HA as a protective shield of nanoparticles from immune recognition and provide a protective layer against cellular toxicity. The use of HA as a coating material can open new avenues to many types of particles in applications that are beyond the scope of RNAi delivery.

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Conflict of interest statement D.P. has financial interest in Quiet Therapeutics, and D.L.M declares no financial interest.

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