ABSTRACT SUMMARY

The PEG length and chemical linkage was systematically varied on a new class of PEGylated polyacidine peptides that bind plasmid DNA with high affinity via polyintercalation. Detailed pharmacokinetic (PK) and biodistribution analysis of $^{125}$I-DNA polyplexes in mice revealed that certain chemical linkages such as thiol-vinyl sulfone (SV) or thiol-acetamide (SA) were unexpectedly metabolically unstable whereas a thiol-maleimide linkage were found to be stable in the circulation. In addition to chemical linkage, PEG length had a dramatic influence on PK and biodistribution. A polyplex possessing a PEG30kDa possesses a long PK half-life and completely avoided liver uptake. Following i.v. dosing of 1 $\mu$g of pGL3 polyplex, a hydrodynamic stimulatory dose was applied at 1 to 9 hours. Liver specific luciferase expression was determined using quantitative bioluminescence imaging at 24 hrs. The results establish important relationships between the PK, biodistribution and gene expression that are strongly influenced by PEG length and chemical linkage. Understanding the interplay between these parameters is a prerequisite to developing i.v. dosed gene delivery systems.

INTRODUCTION

The development of highly efficient nonviral gene delivery systems that mediate expression in animals following i.v. dosing remains a significant challenge. Delivery systems that are optimized in vitro often fail to mediate expression in vivo due to a combination of aberrant pharmacokinetics or biodistribution, lack of intracellular release or trafficking, the inability to penetrate the nucleus, or due to the lack of dose equivalence relative to in vitro gene transfer studies.

To systematically overcome the barriers associated with nonviral gene delivery in vivo with the goal of inventing an efficient nonviral delivery system for the liver, we have adopted a strategy of administering a 1 $\mu$g dose of plasmid DNA (pGL3) polyplex (50 $\mu$l) via the tail vein of mice, followed by a delayed stimulatory hydrodynamic (HD) dose (1.5-2 ml) of saline at delay of up to 1 hr post-DNA delivery, resulting in a luciferase expression equivalent in magnitude to direct hydrodynamic dosing of 1 $\mu$g of pGL3. Improvements in the peptide design included decreasing the number of Acr residue and increasing the spacing of Acr with Lys to arrive at (Acr-Lys$_3$)$_4$-Acr-Cys-PEG5kDa that resulted in stabilized DNA polyplexes in the circulation and a maximal HD-stimulated expression for up to 5 hours.

In the present report, we have examined the influence of PEG length and linkage in relation to the PK, biodistribution, and HD-stimulated gene expression in mice. The results establish that a PEG length of 5kDa linked to (Acr-Lys$_3$)$_4$-Acr-Cys through a thiol-maleimide linkage results in maximal gene expression in vivo. Alternatively, a PEG length of 30 kDa linked via a thiol-maleimide linkage completely avoids liver uptake but still mediates potent gene expression in the liver upon HD-stimulation. This is a fundamental new property for DNA polyplexes dosed i.v., necessary for achieving DNA delivery to tissues outside the liver.

EXPERIMENTAL METHODS

HD-Stimulated Expression (Fig. 1) was performed by tail vein dosing triplicate mice with 1 $\mu$g of DNA or PEGylated polyacidine polyplexes in 50 $\mu$l of HBM (5 mM Heps, 0.27 M mannitol, pH 7.4). At times ranging from 5 min to 9 hrs, an HD-stimulatory dose of normal saline (9 wt/vol% of the body weight) was administered over 5 sec. Bioluminescence Imaging was performed at 24 hrs post HD-stimulation. Triplicate mice were anesthetized by 3% isoflurane, then administered an i.p. dose of 80 $\mu$l (2.4 mg) of D-luciferin (30 $\mu$g/ $\mu$l phosphate-buffered saline). At 5 min following the D-luciferin dose, mice were imaged for bioluminescence (BLI) on an IVIS Imaging 200 Series (Xenogen).

Biodistribution experiments were performed by administering $^{125}$I-pGL3 polyplexes (1.5 $\mu$g), prepared with 0.8 nmol of PEG-peptide per $\mu$g of pGL3 by Dosing via the tail vein in triplicate mice. At times ranging from 5 min to 6 hrs, mice were anesthetized by intraperitoneal

Kevin G. Rice, Sanjib Khargharia, Koby Kizzire, Mark Ericson, and Nick Baumhover
Division of Medicinal and Natural Products Chemistry, College of Pharmacy
University of Iowa, Iowa City, IA, 52242, USA
kevin-rice@uiowa.edu
injection of ketamine (100 mg kg\(^{-1}\)) and xylazine (10 mg kg\(^{-1}\)) and then sacrificed by cervical dislocation. The major organs (liver, lung, spleen, stomach, kidney, heart, small intestine and large intestine) were harvested, rinsed with saline, and the radioactivity in each organ was determined by direct \(\gamma\)-counting and expressed as the percentage of the dose in the organ.

RESULTS AND DISCUSSION

PEGylated polyacridine peptides possessing different linkages between Cys or Pen and PEG were prepared and tested for their ability to form metabolically stable DNA polyplexes in vivo (Fig. 1). Polyplexes (1 \(\mu\)g pGL3) were delivered via a tail dose to mice and at times ranging from 1-9 hours, a blank (saline only) hydrodynamic stimulation was administered. The level of expression by applying HD-stimulation at 1 hour is identical in magnitude to that achieved by direct HD dosing of 1 \(\mu\)g of pGL3. PEGylated peptide polyplexes that showed less metabolic stability (SA, SV and SS) produced lower levels of expression at stimulation times of 3, 4, and 5 hours (Fig 2), whereas more metabolically stable PEGylated peptide polyplexes retained the ability to express at HD-stimulation times of 5 and 7 hours (Fig. 2).

PEG length had a dramatic effect on the pharmacokinetics, biodistribution and gene transfer efficiency of polyplexes. Polyacridine peptides with PEG of 5kDa produced polyplexes that were most active in gene transfer, whereas 2 kDa PEG complete deactivated the gene transfer activity. Biodistribution studies on \(^{125}\)I-DNA PEGylated peptide polyplexes revealed that PEG of 30 kDa completely block liver uptake while retaining HD-stimulated gene transfer activity. The results established a direct correlation between PEG length and liver uptake of polyplexes (Fig. 3).

CONCLUSION

Both PEG length and linkage strongly influence the PK, biodistribution and in vivo gene transfer efficiency of PEGylated polyacridine peptide polyplexes. These high affinity, low molecular weight peptides, bind to DNA by a combination of polyintercalation and ionic inaction, resulting is stable polyplexes that circulate in blood and are stimulated with HD to mediate a high level of expression. Using this approach we found that SA and SV linkages were metabolically unstable, and that 30 kDa PEG allowed polyplexes to avoid liver uptake.

REFERENCES


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