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Toxicogenomics of non-viral drug delivery systems for RNAi: Potential impact on siRNA-mediated gene silencing activity and specificity ☆

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Abstract

RNA interference (RNAi) is an evolutionary conserved cellular process for the regulation of gene expression. In mammalian cells, RNAi is induced *via* short (21–23nt) duplexes of RNA, termed small interfering RNA (siRNA), that can elicit highly sequence-specific gene silencing. However, synthetic siRNA duplexes are polyanionic macromolecules that do not readily enter cells and typically require the use of a delivery vector for effective gene silencing *in vitro* and *in vivo*. Choice of delivery system is usually made on its ability to enhance cellular uptake of siRNA. However, recent gene expression profiling (toxicogenomics) studies have shown that separate from their effects on cellular uptake, delivery systems can also elicit wide ranging gene changes in target cells that may impact on the 'off-target' effects of siRNA. Furthermore, if delivery systems also alter the expression of genes targeted for silencing, then siRNA activity may be compromised or enhanced depending on whether the target gene is up-regulated or down-regulated respectively. Citing recent examples from the literature, this article therefore reviews the toxicogenomics of non-viral delivery systems and highlights the importance of understanding the genomic signature of siRNA delivery reagents in terms of their impact on gene silencing activity and specificity. Such information will be essential in the selection of optimally acting siRNA-delivery system combinations for the many applications of RNA interference.

Keywords: Genocompatibility; siRNA; RNA interference; Gene silencing; Off target effects; Polymer genomics; Gene expression; Drug delivery; Carriers; Non-viral vectors

Contents

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1. Introduction

Post-transcriptional gene silencing by RNA interference (RNAi) appears a promising new approach for the targeted inhibition of gene expression in cell culture and *in vivo*. As such it represents a promising new technology for drug target validation, studying functional genomics and as potential therapeutic agents for diseases of a genetic aetiology (for review see [1–20]). Similar to its predecessors, such as antisense oligonucleotides, ribozymes and DNAzymes (for review see [21–23]), RNAi is a process by which a specific messenger RNA (mRNA) is targeted for degradation as a means of inhibiting the synthesis of the encoded protein. The phenomenon was first reported in plants [24] and termed Post Transcriptional Gene Silencing (PTGS) prior to the discovery of a related process in a wide range of eukaryotic organisms including *Caenorhabditis elegans*, *Drosophila melanogaster*, and mammalian cells (including human cell lines) [25–27]. The alternative natural RNAi mechanism involves the use of microRNA (miRNA) to silence endogenous gene expression (for reviews see [28–31]). miRNAs are a conserved class of non-coding RNAs that naturally negatively regulate gene expression post-transcriptionally by sharing, to some extent, the same cellular biochemical pathways as siRNA [32–34] though some significant differences in the mechanism are becoming apparent [32]. Although their endogenous biological roles are largely unknown, naturally occurring miRNAs are increasingly thought to be important in diseases such as cancer and viral infections. It is therefore thought the miRNAs represent a new drug therapy paradigm for the treatment of these and potentially many other diseases. For a further discussion on miRNAs, the reader is referred to focused reviews on this topic [28–32].

The evolutionary basis of RNAi appears, at least in part, to be an anti-viral defence mechanism though some viruses have now developed RNAi suppressor molecules as a counter-defence strategy [35,36]. It is also thought to be important in silencing mRNAs that are overproduced or translationally aborted [32], guarding the genome from disruption by transposons ("jumping genes") [37,38], and may contribute to genomic imprinting [39,40] or help in defining tissue-specific gene expression patterns by modulating DNA conformation [41].

The RNAi response is triggered by the presence of double stranded (ds) RNA in cells. The dsRNA is degraded into short double stranded fragments (~21–23mer long) termed short interfering (si) RNA by an RNAse III type enzyme Dicer. The siRNA generated is unwound and a single strand enters the RNA induced silencing complex (RISC) [42]. The incorporated antisense strand acts as a guide for the RISC complex to selectively degrade the complementary mRNA. Unfortunately, long double stranded RNA, when present intracellularly in mammalian cells, leads to the initiation of the anti-viral interferon response and global protein expression shutdown. However, this response can largely be avoided by delivering the shorter length siRNA exogenously and/or by avoiding certain RNA sequence motifs that are known to induce an immune response through interaction with specific toll-like receptors [43–51]. Indeed, there is now a growing interest in the proactive design and use of immunostimulatory RNA constructs for potential therapeutic use in a manner analogous to the use of immunostimulatory CpG oligonucelotides (for reviews see [50,52–56].

Provided some basic design rules are adhered to (*e.g.* [5,19,57]) generally siRNA appears to be well tolerated in *in vitro* and *in vivo* models. Thus, the intracellular delivery of siRNA, the key intermediary of RNAi, can elicit a potent knockdown of the desired protein in the absence of an immune response. Indeed, many studies have now demonstrated the effectiveness of siRNA molecules in cell culture and in animal models and the technology is widely used in experimental biology and medicine. RNAi has had a tremendous impact on the biological sciences and for this reason it was hailed as "breakthrough/molecule of the year" in December 2002 by *Science* Magazine. This was followed in late 2006, just about 8 years or so after their discovery of RNAi, that Andrew Fire and Craig Mello received the Nobel Prize for Medicine or Physiology (see also [58]). This truly has been a meteoric rise in the acceptance and use of this new gene silencing technology. Indeed, there are several clinical trials ongoing or planned for taking siRNA into the clinic in the treatment of important diseases such as macular degeneration, cancer, HIV and respiratory diseases (see Table 1).

The first ever human clinical trial with siRNA was conducted by Acuity Pharmaceuticals in late 2004 in patients suffering from age-related macular degeneration (AMD). Local intravitreal delivery to the eye of siRNA (Cand5) targeting the vascular endothelial growth factor (VEGF) was designed to prevent the overgrowth of new blood vessels in the 'wet' form of AMD. This molecule has now reached Phase 2 clinical evaluation with preliminary results indicating that dose-related benefits being observed with respect to several clinical endpoints such as near vision and lesion size. Cand5, as of early 2006, is also being evaluated in separate Phase 2 trial for efficacy in diabetic macular oedema. Sirna Therapeutics entered their fisrt siRNA (Sirna-027) targeting the VEGF receptor for the treatment of the same disease shortly after. Preliminary data from the trial suggested that SiRNA-027 administered as a single intravitreal injection at doses up to 800 μm was well tolerated by patients and improvements in the visual acuity of a sub-set of subjects was also observed. These encouraging safety profiles in man have led other companies to enter siRNA into clinical trials. For example, Alnylam

Table 1
Examples of ongoing or planned clinical trials for siRNA mediated RNA interference

Company	SiRNA product	Disease state	Status
Acuity Pharmaceuticals	Cand5	Age-related macular degeneration (AMD)	Phase 2
	Cand5	Diabetic macular edema	Phase 2
Sirna Therapeutics	Sirna-027	AMD	Phase 1
	Sirna-034	Hepatitis C	Preclinical
Alnylam	ALN-RSV01	RSV lung infection	Phase 1
	Unknown	Pandemic Flu	Preclinical
Atugen	RTP-801i	AMD	Phase 1
	PFTi	Acute renal failure	Preclinical