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REVIEW

DNAzymes and cardiovascular disease

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Gene silencing techniques are gaining increasing popularity in the literature, both as a tool for unravelling gene function and to potentially deliver therapeutic benefit, especially in the context of cardiovascular disease. Gene-specific catalytic DNA molecules, or DNAzymes, have shown promise in ameliorating the effects of myocardial ischaemia reperfusion injury and in-stent restenosis in various animal models, demonstrating that these agents may be useful in a clinical setting. A review of the recent advances in the use of DNAzymes in treating cardiovascular disease is therefore essential given the increasing clinical burden of cardiovascular disease worldwide. We have thus sought to firstly provide background into the construct and mechanism of action of DNAzymes, with a discussion of recent improvements in design. Secondly, we have examined the effects of DNAzyme-mediated gene inhibition in *in vitro* studies of both endothelial and smooth muscle migration and proliferation, as well as *in vivo* models of acute myocardial infraction and neointima formation. Lastly we compare DNAzymes with other gene silencing tools and discuss issues involved in successfully delivering these drugs in a clinical setting.
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Abbreviations: AP-1, activator protein-1; ASK1, apoptosis signal-regulated kinase-1; Egr-1, early growth response factor-1; PAI-1, plasminogen activator inhibitor-1; SMC, smooth muscle cells; VDUP1, vitamin D3 upregulated protein-1

Background

Despite improved pharmacotherapies and mechanical treatments, cardiovascular disease remains a principal cause of morbidity and mortality worldwide, with every likelihood that this burden will increase (Raymond *et al*., 2006; Fox *et al*., 2007; Mark *et al*., 2007). Although gene therapies in the broad sense were proposed over two decades ago, there is now increasing interest in specific gene targeting using small molecule nucleic acid-based techniques (Skarlatos, 2007; van Rooij and Olson, 2007). Numerous approaches are being suggested, based partly on novel technologies which are becoming rapidly available, but also in response to our increasing understanding of the key genes that are responsible for cardiovascular function and dysfunction (Santiago *et al*., 2007; van Rooij and Olson, 2007).

In the setting of this rapidly changing field, this review therefore discusses recent key advances in gene-targeting catalytic DNA molecules, or DNAzymes, in the context of cardiovascular disease.

Drug and molecular target nomenclature conforms to the British Journal of Pharmacology Guide to Receptors and Channels (Alexander *et al*., 2007)

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DNAzymes

DNAzymes are catalytic molecules comprising a single strand of deoxyribonucleotides, with the ability to bind and cleave RNA. They do not occur naturally but were instead developed through an *in vitro* selection process. A library of chimeric molecules was generated, consisting of a single ribonucleotide embedded with fixed primer domains, a random sequence of 50 oligonucleotides and a 5′ biotin moiety (Breaker and Joyce, 1994). Following streptavidin binding, alkali denaturation removed the nonbiotinylated strand. Cleavage of the RNA phosphoester in the presence of divalent metal cation cofactor yielded a catalytically active population in the streptavidin column eluant, which was amplified with PCR and added back to the next round of selection. This approach eventually yielded high enough quantities of selected DNA fractions that were examined for cleavage by electrophoresis, before cloning and sequencing (Breaker and Joyce, 1994; Sun *et al*., 2000).

10–23 DNAzymes Associate

This '10–23' DNAzyme was initially described by Santoro and Joyce (1997, 1998) and was so named as it was derived from the 23rd clone from the 10th round of PCR. The 10–23

DNAzyme is composed of a catalytic domain of 15 deoxyribonucleotides flanked by two substrate recognition arms. It will cleave any RNA substrate between an unpaired purine (A, G) and a paired pyrimidine (U, C) in the presence of Mg^{2+}. The 10–23 DNAzyme has excellent catalytic efficiency, the rate of which is determined by the rate of association between DNAzyme and RNA substrate rather than by the release of cleavage product. As the stability of the DNA–RNA heteroduplex is necessary for DNAzyme catalytic efficiency, choosing an accessible cleavage site within the secondary structure of the mRNA target is necessary for optimal DNAzyme activity (Santoro and Joyce, 1998; Khachigian, 2000). The general structure of the 10–23 DNAzyme (Santoro and Joyce, 1997, 1998) is shown in Figure 1.

The 10–23 DNAzyme is sensitive to base-pairing mismatches in the recognition arms thereby displaying strong substrate specificity, while the length of the recognition arms influences catalytic rate, which is dependent on the target sequence (Santoro and Joyce, 1997). For example, a 10–23 DNAzyme designed to target c-myc RNA had improved catalytic efficiency with 8–9 base-pair arms compared with 7 base-pair arms (Sun *et al*., 1999). This 10–23 DNAzyme has the additional modification of a 3–3′ inverted thymidine at the 3′ end of the DNAzyme, which reduces exonuclease degradation (Bhindi *et al*., 2007). The 10–23 DNAzymes are active at physiological pH and Mg^{2+} concentration, the latter of which is necessary for activity, possibly by stabilizing the DNA-RNA heteroduplex or by maintaining the DNAzyme in its active conformation (Khachigian, 2000). Most DNAzymes studies have used the '10–23' catalytic core as the starting point for cleavage of a specific target sequence, with the view to producing biological effect as will be discussed later in this review.

Further modifications

As mentioned, DNAzyme stability may be improved by the addition of a 3–3′ inverted nucleotide at the 3′ end of the DNAzyme. This modification can increase the stability of the molecule and has been shown to extend DNAzyme half life from 70 min to 21 h in human serum (Dass *et al*., 2002). DNAzymes with this modification can also remain functionally intact for at least 24–48 h after exposure to serum, compared with their unmodified counterparts (Santiago *et al*., 1999c; Dass *et al*., 2002). Phosphorothioate linkages—previously employed to protect ribozymes and antisense oligonucleotides from endogenous nucleases—have also been proposed as modifications for DNAzymes (Lu *et al*., 2005). The introduction of phosphorothioate modifications, however, adds a negative charge thereby decreasing the binding affinity for mRNA and thus catalytic potency (Cairns *et al*., 2002; Dass *et al*., 2002). Phosphorothioate linkages have also been associated with toxicity (Wahlestedt *et al*., 2000), immunological disturbance (Fluiter *et al*., 2003) and protein interactions, resulting in sequence-independent or nonspecific effects (Guvakova *et al*., 1995; Rockwell *et al*., 1997).

A development in DNAzyme design is that of the locked nucleic acid (LNA). LNA-DNAzymes have LNA monomers introduced into the usual hybridizing arms, increasing the melting temperature of the molecule and enhancing binding affinity by conformational constraint placed on the sugar ribose ring. LNA-modified DNAzymes have higher catalytic rates and sensitivity to target than that of unmodified DNAzymes (Fahmy and Khachigian, 2004). Our group provided the first functional demonstration of LNA-modified DNAzyme efficacy in a biological setting (Fahmy and Khachigian, 2004). However, higher melting temperature can also result in slower reaction rates and a five-fold difference in production cost compared with unmodified DNAzymes may mean these molecules present as a less attractive therapeutic option.

A further, more recent, attempt to improve DNAzyme stability is the introduction of hairpin DNAzymes, where stem-loop hairpins are added to the end of the substrate-binding arms. Hairpin DNAzymes display resistance to nucleolytic degradation for up to 3 days after transfection and produce better gene knockdown than non-hairpin DNAzymes with the same catalytic domain. No non-specific effects or cytotoxicity have been observed to date with these agents (Abdelgany *et al*., 2007).

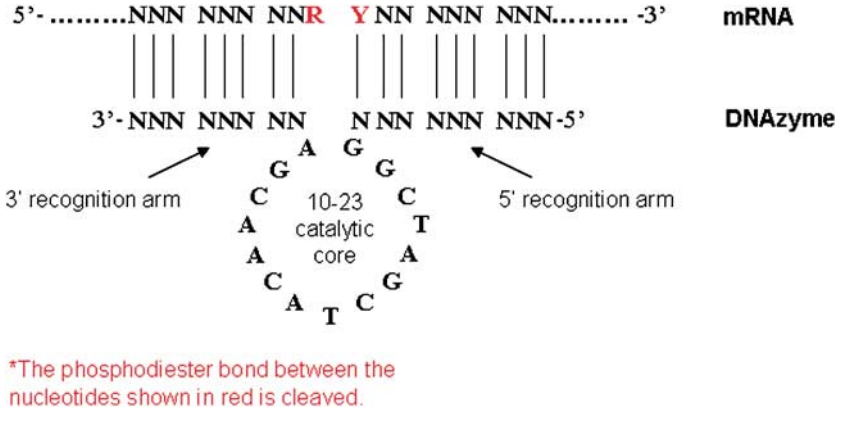

Figure 1 DNAzyme structure.

Table 1 DNAzymes and *in vivo* cardiovascular pathologies.

Target	*DNAzyme*	*Model*	*Effect*	*References*
Egr-1	**ED5** 3′-TAGCAGGTCCAGCAACATCCATCGGACCGGC GCC-5 (inverted 3′ T)	Rat and pig ballon catheter injury	Inhibition of neointima formation	Santiago *et al.*, 1999a, b, c; Bhindi *et al.*, 2006
		Rat myocardial ischaemia-reperfusion injury	Attenuation of infarct size and inflammatory mediators	
c-jun	**Dz13** 3′-TGTTGCGGAAGCAACATCGATCGGAAGGAGGGC-5′ (inverted 3′ T)	Rat carotid artery ligation	Inhibition of neointima formation	Khachigian *et al.*, 2002
TNF-α	**Active TNFα Dz** 3′-TsTsTsCsCsTsGsTsGsGAGCAA CATCGGAsCsTsCsGsTsG-5′	Rat AMI by LAD ligation	Increased cardiac output	Iversen *et al.*, 2001
PAI-1	**E2** 3′-TACGTCTAAGCAACATCGATCGGAGAA GTCG-5′ (inverted 3′ T)	Rat AMI by LAD ligation	Enhanced neovascularization, cardiomyocyte regeneration and function recovery with angioblast coinjection	Xiang *et al.*, 2005a, b
VDUP1	**E4** 3′-TCGAGTTAGAGGCAACATCGATCGGAC CACTAC-5′ (inverted 3′ T)	Rat AMI by LAD ligation	Decreased apoptosis and collagen expression, increased function	Xiang *et al.*, 2005a, b

Abbreviations: AMI, acute myocardial infarction; Egr-1, early growth response factor-1; LAD, left anterior descending coronary artery; PAI-1, plasminogen activator inhibitor; TNF-α, tumour-necrosis factor; VDUP1, vitamin D3-regulated protein-1.

DNAzymes in the investigation of specific cardiovascular diseases

DNAzymes have two potentially broad uses in biology, both achieved by gene downregulation. DNAzymes may first be used as tools to dissect the function of specific genes. They may also have potential therapeutic benefit by suppressing the expression of pathophysiologically active genes. A number of cardiovascular pathophysiologic states would appear suitable for the potential application of DNAzymes—like other gene-silencing approaches—to achieve therapeutic benefit. These conditions are principally where (a) the pathophysiologic process is localized in both time and place, such that local delivery of a potentially biodegradable agent may have the best chance of effect and (b) where the possibility of local drug delivery exists readily in a clinical context. Such processes include restenosis following balloon angioplasty or stent deployment and acute myocardial infarction (AMI). In the case of restenosis postangioplasty or stenting, there is additional advantage that, in the clinical situation, the onset of the process is predictable and initiated by the physician. A further consideration in DNAzyme applicability is the relevance of the target gene. The discussion that follows groups the various DNAzymes according to their target gene, alongside a description of the gene itself (Table 1).

Egr-1 DNAzymes

Early growth response (Egr-1) is a zinc finger transcription factor known to be upregulated by mechanical injury to vascular smooth muscle cells (SMCs) and endothelial cells in a variety of contexts (Khachigian *et al.*, 1996; Silverman *et al.*, 1999; Santiago *et al.*, 1999c). The human Egr-1 promoter has five to six serum response elements, which require appropriate ternary complex factors and serum response factor to form a complex to activate the promoter (Gashler and Sukhatme, 1995). Egr-1 regulates transcription by a number of mechanisms, including its ability to functionally interact with other transcription factors. For example, Egr-1 induction of platelet-derived growth factor-A is mediated by displacement of Sp1 on the platelet-derived growth factor-A promoter (Khachigian *et al.*, 1995) thus stimulating platelet-derived growth factor-A mRNA transcription. Overlapping consensus sites for Egr-1 and Sp1 also appear in the promoter regions of transforming growth factor β1, tissue factor and the adhesion molecules CD44 and intracellular adhesion molecule-1 (Khachigian, 2001). Other Egr-1 targets include fibroblast growth factor (Biesiada *et al.*, 1996), PAI-1 (Liu *et al.*, 1999), tumour-necrosis factor-α (Kramer *et al.*, 1994) and vascular endothelial growth factor receptor Flt-1 (Vidal *et al.*, 2000).

Egr-1 can downregulate itself by binding to its own promoter (Thiel and Cibelli, 2002) or by interactions with Nab1 and Nab2 via an inhibitory domain (Thiel and Cibelli, 2002). Egr-1 activation occurs within minutes (Khachigian *et al.*, 1996) and mRNA and protein levels quickly degrade over several hours (Huang *et al.*, 1999), the latter likely due

to Nab2 expression, which is both rapid and transient in response to agonist (Silverman *et al*., 1999).

Egr-1 in SMC responses to injury

A functional role for Egr-1 in SMCs was initially established by experiments using conventional antisense oligonucleotides directed to the Egr-1 start codon or to a sequence encoding one of the crucial zinc fingers that are required for Egr-1 DNA binding. These oligonucleotides were shown to inhibit the inducible synthesis of Egr-1 protein in a sequence-specific manner and to inhibit DNA synthesis and cell replication (Santiago *et al*., 1999a). These experiments demonstrated that Egr-1 is required for SMC and endothelial cell recovery from mechanical injury *in vitro* (Santiago *et al*., 1999a, b).

A DNAzyme targeting the $A^{816}U$ junction within the start codon of rat Egr-1 was developed. This DNAzyme, denoted as ED5, was designed with 9 + 9 base-pair-recognition arms and carried a 3′–3′-linked inverted thymidine for added stability (see Figure 1). ED5, as expected, cleaved a short synthetic Egr-1 RNA substrate and *in vitro* transcribed mRNA of various lengths, in a sequence-specific manner (Santiago *et al*., 1999c) (Figure 2). Importantly, using a fluorescein-tagged version of this DNAzyme it was demonstrated that the molecule localized within SMC nuclei. ED5 inhibited endogenous Egr-1 mRNA and protein synthesis and blocked SMC proliferation in response to serum (Santiago *et al*., 1999c). Like Egr-1 antisense oligonucleotides, ED5 suppressed the reparative response to a scraping injury *in vitro*.

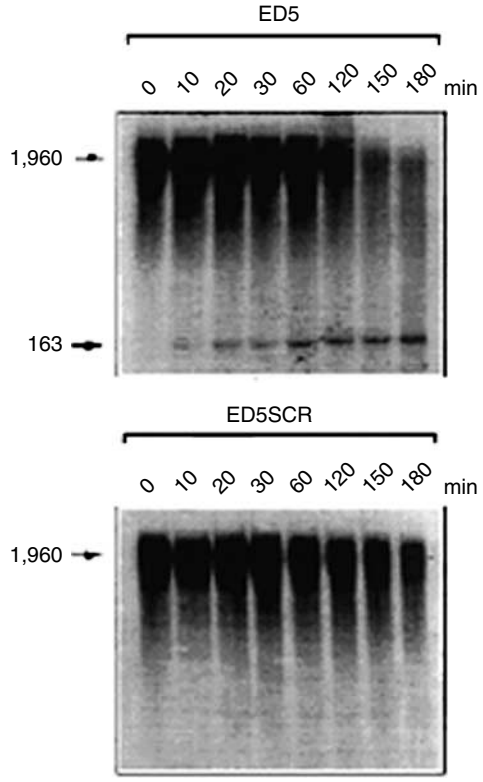

Figure 2 Time and sequence effects of DNAzyme. Autoradiogram of an *in vitro* 1960 nucleotide Egr-1 transcript is shown in both panels, after exposure to active Egr-1 DNAzyme (ED5, upper panel) or its scrambled control (ED5SCR, lower panel). Time-dependent diminution of signal is indicated, with the gradual appearance of the expected 163-nucleotide fragment, only after exposure to ED5. (From Santiago FS *et al*. Nature Medicine 1999; 5:1264-9, permission pending).

As SMC proliferation is a characteristic of neointima formation following angioplasty, ED5 was tested *in vivo* using a balloon catheter injury model of neointima formation in rats. Fluorescein-tagged DNAzymes localized within the rat carotid artery wall after adventitial delivery using a pluronic gel. ED5 inhibited the upregulation of Egr-1 protein in the media of the vessel wall, shortly after balloon injury, and inhibited neointima formation 14 days after adventitial delivery (Santiago *et al*., 1999c). This study was the first to demonstrate DNAzyme efficacy *in vivo*. Similar delivery methods revealed reduction of neointima formation following simple ligation of the rat carotid artery (Lowe *et al*., 2002).

DNAzymes targeting human Egr-1 have likewise been developed and locally delivered by other means to achieve suppression of neointima proliferation in other similar contexts. Intraluminal delivery of DNAzyme, suffused into the coronary vessel wall, was achieved during coronary stenting in pigs following confirmation of specificity in porcine SMCs. This mode of delivery resulted in reduced neointima formation (Lowe *et al*., 2001). The same DNAzyme also reduced neointima formation when added to media in an *ex vivo* human mammary artery explant model (Lowe *et al*., 2001).

While the above data demonstrate beneficial effect of Egr-1 suppression in reducing neointima formation, interestingly, serum-induced endothelial cell proliferation and migration is also inhibited by other Egr-1-targeting DNAzymes (Fahmy *et al*., 2003). While initially an appealing result, in the context of developing strategies for intravascular neointimal proliferation following stenting, these DNAzyme effects on endothelial cells have the potential to cause detrimental effects. Lack of complete endothelial cell layer coverage of coronary stents has been proposed as a risk factor for late stent thrombosis, particularly after drug-eluting stent implantation (Finn *et al*., 2007). There remains the possibility that reduced endothelial cell coverage could occur with DNAzyme therapy, although local delivery site, dose and drug kinetics are all likely to play important roles in any such detrimental effects.

Egr-1 in ischaemia–reperfusion injury

Early growth response factor-1 has also been recognized to play a role in the response to ischaemia–reperfusion injury in a variety of other cell types and organ systems. Outside the cardiovascular system, Egr-1 has been implicated in ischaemia–reperfusion responses in the lung (Yan *et al*., 2000), intestine (Chen *et al*., 2004) and kidney (Bonventre *et al*., 1991). Specifically within the cardiovascular system, Egr-1 upregulation has been observed in the myocardium in response to permanent coronary ligation in the mouse (Lyn *et al*., 2000) and following brief coronary occlusion in

the pig (Brand *et al*., 1992). Any functional role such Egr-1 upregulation might play had not been investigated until recently.

The hypothesis that Egr-1 activation could contribute to the pathologic response in myocardial ischaemia–reperfusion injury was tested by the application of ED5 to a rat model of temporary left coronary artery occlusion. Egr-1 mRNA and protein expression were both elevated in this model and were selectively inhibited by prior delivery of ED5 (Bhindi *et al*., 2006). Importantly, infarct size decreased 50% with ED5 but not scrambled DNAzyme or vehicle controls. Inhibition of neutrophil infiltration and intracellular adhesion molecule-1 protein were also observed, suggesting that Egr-1 is involved in the inflammation response to ischaemia–reperfusion injury, possibly by intracellular adhesion molecule-1 as a primary downstream target (Bhindi *et al*., 2006). These studies were the first to demonstrate the importance of Egr-1 targeting within the myocardium as a possible therapeutic option. As Egr-1 targeting DNAzymes are also known to inhibit angiogenesis by downstream repression of fibroblast growth factor-2 (although not vascular endothelial growth factor) (Fahmy *et al*., 2003), there is similarly the potential for reduced angiogenesis in this context, making this an area of active investigation.

c-Jun-targeting DNAzymes

The proto-oncogene c-Jun, a component of the activator-protein-1 (AP-1) transcription factor, has been the subject of much recent interest in the DNAzyme field. The role of c-Jun in a wide variety of disease processes has been unravelled using Dz13, a DNAzyme directed against human c-Jun, which also has an inhibitory role in porcine and rat cells (Khachigian *et al*., 2002). Dz13 inhibited human, porcine and rat SMC proliferation *in vitro* and suppressed neointima formation in rats subjected to carotid artery ligation (Khachigian *et al*., 2002). Dz13 also suppressed proliferation and migration of human microvascular endothelial cells *in vitro* (Zhang *et al*., 2004), which suggested an important regulatory role for c-Jun in angiogenesis. The protein content and proteolytic activity of matrix metalloproteinase-2, an enzyme that commonly degrades extracellular matrix proteins and basement membrane, was also repressed by Dz13 thus implicating matrix metalloproteinase-2 as a downstream transcriptional target of c-Jun in this setting. Further evidence for the angiogenic role of c-jun was revealed when vascular endothelial growth factor-induced neovascularization in rat corneas and hypoxia-induced retinal neovascularization were both inhibited by *in vivo* delivery of Dz13 (Zhang *et al*., 2004; Fahmy *et al*., 2006), and similarly, tumour growth in mice (Zhang *et al*., 2004). Extensive studies in murine models of inflammation showed that Dz13 inhibited vascular permeability, endothelial-monocytic adhesion, leukocyte adhesion and extravasation, and neutrophil infiltration (Fahmy *et al*., 2006). Among the downstream targets of Dz13 are E-selectin, VCAM-1, intracellular adhesion molecule-1 and VE-cadherin (Fahmy *et al*., 2006).

Other DNAzymes

Tumour-necrosis factor-α is associated with atherosclerosis (Libby *et al*., 1995), AMI (Ono *et al*., 1998) and heart failure (Torre-Amione *et al*., 1996; Yue *et al*., 1998). Rats implanted with osmotic pumps secreting a tumour-necrosis factor-α-specific DNAzyme for 2–4 weeks post-AMI had improved cardiac output and lower heart weights than inactive DNAzyme-treated controls (Iversen *et al*., 2001). No toxicity was observed and extracted DNAzyme still retained cleavage activity even after 4 weeks *in vivo* treatment (Iversen *et al*., 2001). Another marker of cardiovascular disease is PAI-1, levels of which are elevated in patients with restenosis, atherosclerosis and AMI. Rat PAI-1 DNAzyme inhibited transforming growth factor β-mediated PAI-1 stimulation in rat endothelial cells (Xiang *et al*., 2004). Injection of this DNAzyme into the peri-infarct zone following AMI in the rat resulted in decreased PAI-1 expression up to 2 weeks post-treatment. Improved neovascularization of the infarcted tissue was demonstrated by increased transmigration of tagged human adult bone marrow-derived endothelial progenitors (angioblasts) and increased capillary density of human origin, indicating that the inhibitory effect of PAI-1 on plasmin generation and adhesion mediators had been countered by PAI-1 DNAzyme treatment (Xiang *et al*., 2004). Further work by this group with PAI-1 DNAzyme delivery to infarcted mouse hearts revealed not only PAI-1 inhibition and improved neovascularization, but also decreased apoptosis in the peri-infarct region and improved cardiac function, as measured by myocardial ejection fraction (Xiang *et al*., 2005a).

DNAzymes have also been used to study proteins associated with increased oxidative stress due to myocardial ischaemia, in particular the vitamin D3-upregulated protein-1 (VDUP1). VDUP1 inhibits interaction between thioredoxin and apoptosis signal-regulated kinase-1 (ASK1). Transfection of a rat-specific DNAzyme targeting VDUP1 into the rat myoblast line H9c2 decreased VDUP1 mRNA levels and ASK1 activity. Lower apoptotic levels were observed in transfected cells challenged with H_2O_2 compared with control (Xiang *et al*., 2005b). Infarcted nude rats had elevated VDUP1 at 2 weeks post–injury, which was abrogated by VDUP1 DNAzyme delivery at injury. More significantly, DNAzyme treatment decreased infarct size and improved ejection fraction, although it was noted that higher concentrations of DNAzyme had an adverse effect on mortality (Xiang *et al*., 2005b). As ASK1 phosphorylation was decreased *in vivo*, it was suggested that complete inhibition of ASK1 could be detrimental due to the ubiquitous role of ASK1 as a homeostatic regulator.

Advantages and limitations of DNAzymes compared to other nucleic acid-based agents

DNAzymes are the subject of increasing interest, in that they demonstrate a number of potential advantages over certain other available nucleic acid-based agents (Fiammengo and Jaschke, 2005; Eckstein, 2007). Synthesis is relatively inexpensive, and the molecules are stable relative to ribozymes

and antisense molecules. Deoxyribonucleotides are more resistant to nuclease activity than ribonucleotides, and modifications, such as the 3–3′ inverted nucleotides and hairpin structures, further increase stability both *in vitro* and *in vivo*. As already noted, disadvantages of phosphorothioate linkages and aptamers are avoided by all DNA-based phosphodiester-linked DNAzymes. DNAzyme activity is influenced by the sequence of the binding arms, conferring gene selectivity. Use of scrambled arms as controls allows demonstration of target sequence specificity.

A number of other gene knockdown techniques have been explored, including antisense, ribozymes, decoys, short interfering RNAs (siRNAs) and other techniques, reviewed extensively elsewhere (Bhindi *et al*., 2007). Antisense molecules have been widely evaluated, and their limitations well documented, such as nonspecific protein interactions due to the formation of G-quartets (Stein, 1997). Ribozymes share structural similarity to DNAzymes, with a central catalytic core, but with the limitation of RNA components which are more susceptible to degradation (Bhindi *et al*., 2007). siRNAs have received wide attention recently and are currently being trialled as therapy in retinopathies, cancer, cardiovascular disease and inflammatory disorders (Crooke, 2004). Strategies have evolved to increase siRNA specificity, by complexing the molecule with peptides or ligands to allow better tissue targeting (Bhindi *et al*., 2007). siRNAs inhibiting Egr-1 expression have been developed, and although differences in mechanism make direct comparisons of efficacy problematic for these specific siRNAs, there are suggestions that potency may be greater for siRNA compared with DNAzyme (Fahmy and Khachigian, 2007).

Optimal stability and maintenance of cleavage activity are vital for future clinical application of these drugs. Some of the challenges facing the successful transition of DNAzymes into therapeutic agents are similar to those of other nucleic acid-based therapies, namely, delivery, cellular uptake and bioavailability. The '10–23' DNAzymes (approximately 10 kDa) require cationic lipid transfection reagents for intracellular delivery, and these reagents can be problematic in that they can cause nonspecific effects (Cairns *et al*., 2002).

Future directions

DNAzyme development in the cardiovascular field is likely to follow two paths. Firstly, DNAzymes having demonstrated *in vivo* biologic efficacy (for example, those targeting Egr-1, c-Jun and PAI-1, in the vasculature and myocardium) are likely to see continued development towards clinical application. However, the challenge of drug delivery, at every level from cell and organ access to the choice of lipophilic vehicle agent, will need careful consideration. Secondly, new pathophysiologically relevant genes will serve as novel candidate targets. Given the breadth of clinically relevant cardiovascular diseases and the advances made to date with DNAzymes toward therapeutics, this is likely to remain an area of growing interest.

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Conflict of interest

The authors state no conflict of interest.

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