Can aptamers help with cardiovascular disease?

Cardiovascular disease (CVD) is the leading cause of death and disability worldwide. It accounts for approximately one third of all deaths globally (Shattat, 2016) and represents a massive financial burden on health services. Innovation and collaboration are required to develop improved therapeutics and diagnostic applications, in which aptamers may play a crucial role.

What is Cardiovascular disease?

Cardiovascular disease is commonly defined as any disease affecting the heart and/or vascular network and can manifest in several different ways, including angina, heart attacks and stroke. Despite the different outputs, the common pathology of CVD is atherosclerosis – otherwise known as a narrowing of blood vessels by build-up of fatty plaques (Wong, 2014). Over time, these plaques can rupture and release toxins into the bloodstream resulting in platelet activation, coagulation and clot formation. Taken together, these events block vessels within the heart, brain and other organs leading to localised cell death.

Whilst much of the diagnosis regarding CVD relies on various imaging techniques of the vessels, CVD related biomarkers can also provide important insights for clinicians. Measurement of CVD markers can be used for assessment of disease progression as well as selecting the best course of treatment. However, only markers to detect heart failure have reached clinical approval in recent years. As a result, an improved effort is required to identify novel biomarkers associated with CVD and to translate these findings into new diagnostics and therapies.

Identifying new biomarkers with aptamers

The need for novel biomarkers has caused the development for novel aptamer based biomarker discovery platform, including Aptasort by Aptamer Group. Through rounds of selection and counter-selection, aptamers can be raised to previously unknown biomarkers within a specific population. Examples of novel biomarkers include cell surface receptors, in which expression changes with disease progression and secreted proteins in plasma.

Once aptamers have been raised to the previously unknown material, the aptamer can be utilised for simple ‘immunoprecipitation’ like experiments, coupled with mass-spectroscopy to identify the protein of interest.

Although most cardiac biomarkers in clinical use detect heart failure, the cardiovascular field is in great need of biomarkers that span the cardiovascular disease spectrum from developing atherosclerosis to late-stage disease. Aptamers can therefore provide a novel alternative to detect
more precise risk associated biomarkers to enhance patient stratification and ultimately effective treatment.

**Diagnosing CVD using aptamers**

Several biomarkers have already been characterised for CVD, including myoglobin, C-reactive protein and L-homocysteine. Detection of these analytes using aptamers has been performed in a range of different formats including microfluidic devices (Wang et al. 2014), coupling to SPR platforms (Yang et al. 2014) and nanoparticle release assays (McKeague et al. 2013). Anti-thrombin aptamers have also been frequently used for proof-of-principle demonstrations of various detection methods and as model aptamers to explore modes of aptamer-based biosensors (Deng et al. 2014). The versatility of aptamer conjugation has given rise to a range of different diagnostic readouts, including electrochemical, fluorescence, chemiluminescence, and colometric assays (Chen and Yang, 2015).

More recently, there has been considerable interest in finding diagnostic and prognostic biomarkers that can be detected in blood to predict early onset CVD risk. Of these, C-reactive protein (CRP) is the best known biomarker, followed by cardiac troponin I or T, myoglobin, lipoprotein-associated phospholipase A₂, interleukin-6, interleukin-1, oxidised low-density lipoprotein, myeloperoxidase, tumour necrosis factor alpha and soluble LOX-1 (Hayashida et al 2005). Significant effort is being made by biomedical researchers to validate these targets for use in the clinic.

**Aptamers in cardiovascular therapeutics**

The major limitations of existing therapies/therapeutic agents in CVD include (Becker et al. 2009):

1. Target non-selectivity.
2. Variable onset and offset of pharmacodynamic effects.
3. A narrow efficacy-safety profile.
4. The absence of a safe and reliable platform for either accurate titration or active reversibility.

Anticoagulation and anti-platelet agents have become areas of research for novel aptamer based therapies. This is due to the fact that aptamers present faster tissue penetration and wider applicability with the opportunity for simple base modifications to improve functionality as anti-coagulation agents (Jayasena, 1999).
The coagulation cascade is a complex series of biochemical reactions that results in the polymerisation of fibrin and formation of platelet/fibrin homeostatic plugs to prevent blood loss. Elevated activation of the coagulation pathway can result in increased risk of thrombosis and therefore is closely monitored and currently treated with drugs such as warfarin and heparin (Woodruff, 2013). Specific and potent aptamers have been developed to various targets involved in the coagulation cascade including:

- Thrombin
- Factor Xa
- Factor IXa*
- Factor VIIa (otherwise known as Tissue Factor)*

*The factor IXa aptamer in clinical development acts as a potent anticoagulant in patients by inhibiting factor X binding to the factor IXa-factor VIIIa complex (Sullenger, Woodruff, and Monroe, 2012).

A problem with current anti-coagulants, including dabigatran and rivaroxaban is that they are simply too effective and can increase risk of bleeding with use. Early phase clinical studies with the anti-factor IXa aptamer have shown that the ability of the aptamer to block factor IXa activity outweighs any negative consequences of blocking antithrombin inhibition (Sullenger, Woodruff, and Monroe, 2012). This suggests that the factor IXa aptamer acts as a potent anticoagulant in patients by inhibiting factor X binding to the factor IXa-factor VIIIa complex. Moreover, effective ‘antidotes’ can be produced for aptamer therapies by infusion of a complementary oligonucleotide sequence to form dsDNA, allowing fine tuning of therapies.

**Anti-platelet agents**

There has also been interest in targeting specific cellular targets, particularly associated with platelets and reducing platelet activation. The role of platelets in atherosclerotic process and subsequently in the pathophysiology of cardiovascular disease (Papapanagiotou et al 2016) has resulted in many different research programs aimed to target these cells.

After the adhesion of platelets on the injured vascular endothelium and activation, a wide range of molecules stored in platelets granules such as chemokines, pro-inflammatory molecules, and other biological response modulators are released. This accelerates interaction among platelets, endothelial cells, and leukocytes. These interactions establish a localized inflammatory response that promotes the atherosclerotic process (Papapanagiotou et al 2016).
Several aptamers have been raised against molecules targeting platelets. These include:

- P-selectin (CD62P)
- Von Willebrand factor and GPIb-V-IX
- Thrombin and PAR1 / PAR4 receptors

The aim of aptamers targeting these receptors is to prevent platelet adhesion and activation and to stop cells from clumping together. The most extensive work has been undertaken looking at thrombin – a serine protease which works to activate platelets and convert fibrinogen to fibrin to aid in clot formation. Inhibition of thrombin in vivo using aptamers prevents thrombin activity by competing with the active site (Wang et al 2011). A short-acting aptamer which binds to thrombin was tested in a canine cardiopulmonary bypass (CPB) model to determine its anti-coagulant efficacy and potential as a substitute for heparin in CPB and other clinical situations (DeAnda et al 1994). It was found that this aptamer was both safe and effective as an anti-coagulant in the canine CPB model with predictable pharmacokinetics.

**Conclusions**

It is evident that over the past few years, much progress and interest has taken place in the development of therapeutic aptamers including anticoagulants, antithrombotic and anti-adhesives. The Aptasort technology has potential to accelerate development of these tools and therapies, particularly in regards to development of specific early disease onset biomarkers. From anti-platelet to intracoronary stent coating, aptamers possess enormous potential for biomedical applications in treating cardiovascular diseases.

**The Aptamer Group**

Aptamer Group takes a high-throughput approach using liquid handling robotics and dedicated researchers to identify aptamers against novel and significant targets. We are committed to finding the perfect aptamers to your target and use a proprietary selection technique to identify high affinity aptamers with specificity in as short as 3 months.

Aptamer Group’s biomarker discovery, diagnostic and therapeutic divisions aim to conduct further research in the prevention, diagnosis, and treatment of cardiovascular disease. Through our know-how and key collaborators, we are able to help facilitate the development of aptamers as therapeutics or diagnostic devices for your target of interest.

For enquiries, please contact us on info@aptamergroup.co.uk
References:


