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Review Article

Development of Aptamer oligonucleotides as Anticoagulants and Antithrombotics for Cardiovascular Diseases: Current Status

Weibin Li [a,b], Kaiyu Wang [b], Meng Zhao [b], Xiangyue Yang [b], Min Chen [b], Xiaopeng Lan [a,b,*]

[a] Second Military Medical University, Shanghai 200438, China
[b] Institute for Laboratory Medicine, Fuzhou General Hospital of Nanjing Military Command, No 156 North Xi-er Huan Road, Fuzhou City, Fujian Province, 350025, China

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ABSTRACT

Aptamers are short DNA/RNA oligonucleotides selected by a process known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX) based on affinity for target molecules. Since aptamers have several advantages over monoclonal antibodies, such as high specificity and affinity, flexible modification and stability, and lack of toxicity and immunogenicity, they are promising novel diagnostic and therapeutic agents. In this review, we will describe the development of aptamers against thrombin, von Willebrand factor (vWF), factor IX, and factor XII as potential anticoagulants or antithrombotics for cardiovascular diseases, especially those that have entered clinical trials.

Contents

Introduction

Cardiovascular diseases, including acute myocardiac infarction, acute coronary syndromes (ACS),ischemic stroke and venous thromboembolism, cause substantial health care expenditures, morbidity, and mortality worldwide. Antiplatelet drugs and anticoagulants are the most widely used therapeutics for patients at the risk of cardiovascular events. Antiplatelet drugs include cyclooxygenase (COX) antagonist like aspirin, purinergic receptor P2Y, G protein-coupled 12 (P2Y12) platelet receptor antagonism (clopidogrel, prasugrel, ticagrelor), and platelet membrane receptors glycoprotein (GP) IIb/IIIa inhibitors. They are mainly used by patients to treat or prevent ACS and stroke. The major clinical limitation of current antiplatelet drugs is increasing bleeding potential [1–5]. In addition, ticagrelor is associated with dyspnea and bradyarrhythmia [6,7]. Anticoagulants include 1.The factor Xa (FXa) inhibitors (unfractionated heparin, low molecular weight heparin, fondaparinux, apixaban), 2.Thrombin inhibitors such as unfractionated heparin, low molecular weight heparin, recombinant hirudins (bivalirudin, desirudin, lepirudin), argatroban, 3. vitamin K antagonists (warfarin). They are highly effective in prevention and treatment of various thromboembolic disorders. Aside from bleeding potential of above-mentioned drugs, there are some drawbacks of heparin and warfarin, including heparin-induced thrombocytopenia (HIT) and requirements of regular anticoagulant monitoring of international normalized ratio of prothrombin time (PT-INR) and activated partial

* Corresponding author at: Institute for Laboratory Medicine, Fuzhou General Hospital of Nanjing Military Command, No 156 North Xi-er Huan Road, Fuzhou City, Fujian Province, 350025, China. Tel.: +86 1 3805013386.
E-mail address: lanxp@sina.com (X. Lan).

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thromboplastin time (APTT) [8]. In addition, administration of fondaparinux, a low molecular weight heparin, and recombinant hirudins are problematic to patients with severe renal insufficiency. Thus novel antiplatelet drugs and anticoagulants that will not increase the risk of bleeding are urgently needed for cardiovascular disorders.

'Aptamer' is from the Latin 'aptus', meaning 'to fit'. Aptamers are DNA/RNA oligonucleotides generated in vitro based on affinity for target molecules by a process that combines combinatorial chemistry and in vitro evolution, known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX) first reported in 1990 [9,10]. Aptamers are a novel class of oligonucleotides with drug-like properties. They share some of the attributes of monoclonal antibodies but have several advantages over antibodies and other therapeutics. Firstly, low off-rates makes aptamers bind to their target with high affinity and specificity (with K_d values ranging from picomoles to nanomoles). Secondly, aptamers can be easily synthesized in vitro, so the variability between the batches can be avoided and high quality be guaranteed economically. Thirdly, aptamers can be chemically modified on the sugar backbone by 2'-O-methyl and 2'-fluoro substitutions, phosphorothioate or functional groups on either 5' or 3' end to enhance their in vivo stability. In addition, therapeutics aptamers can be delivered with drug molecules, virus, siRNA, liposomes, micelle, polyethylene glycol (PEG), and nanomaterials. Lastly, lack of toxicity and immunogenicity is the most favorable advantage over antibodies [11–13].

Compared to applications of antibodies, aptamer research is still in its starting phase, but making progress at a rapid pace. In 2004, Pegaptanib (Pfizer/Eyetech), an aptamer against human vascular endothelial growth factor (VEGF), was approved by the Food and Drug Administration (FDA) for age-related macular degeneration (AMD) [14]. Thereafter, a variety of other aptamers were developed for different diseases, such as virus infection [15], leukemia [16], tumor [17], and autoimmune disease [18]. In this review, we will focus on aptamers against von Willebrand factor (vWF), thrombin, factor IX and factor XII as potential anticoagulants or antithrombotics for cardiovascular diseases.

Therapeutic Aptamers for Cardiovascular Diseases

Antithrombin Aptamers

Thrombin (coagulation factor IIa) is a serine protease, derivative of activation of prothrombin (coagulation factor II). In the blood coagulation and hemostasis pathway, thrombin can activate factor V, VIII, XI, XIII and convert soluble fibrinogen into insoluble strands of fibrin, which forms fibrin clots [19,20]. Thus thrombin can regulate intrinsic coagulation pathway by activating factor XI. Moreover, prothrombin can activate platelet by binding its anion-binding site, namely exosite 2 to GP Ib-α on the cell membrane of the platelet [21]. In addition, thrombin has some activities similar to growth factor and cytokines and is involved in atherosclerotic plaque formation, injury repair and inflammation.

Presently antithrombin aptamers are the most intensively studied aptamers as both diagnostic and therapeutic agents. The first antithrombin aptamer was reported in 1992 [22]. HD1 has a 15-nucleotide G-quadruplex structure. It inhibits the functions of thrombin including conversion of fibrinogen to fibrin and platelet activation by interacting with exosite 1 of thrombin [23]. Exosite 1 binds ligands such as fibrinogen, factor XI, the cofactors V and VIII and thrombomodulin [24]. HD22 is another G-quadruplex structure antithrombin DNA aptamer, binding to exosite 2 of thrombin. Exosite 2 is involved in the binding site for GP Ib-α-chain of platelet GPIb/IX, the activation of factor V and factor VIII, and mediates heparin binding [25]. However phase 1 clinical trial of HD1 (Archemix and Nuvelo) as an anticoagulant during bypass surgery failed to show its efficacy [26].

HD1-22 is a bivalent of two G-quadruplex structure antithrombin aptamer developed by connecting HD1 and HD22 through a polynucleotidic linker. Müller et al. [27] demonstrated that HD1-22 bond to thrombin with high affinity ($K_d = 0.65$ nM) and occupied both the exosite 1 and exosite 2 without blocking the active centre of the enzyme. Kinetic parameters of surface plasmon resonance (SPR) showed that compared with the monovalent aptamers HD1 and HD22, the affinity of aptamer HD1-22 to human α-thrombin was significantly improved. A series of clotting experiments showed that anticoagulant activity of HD1-22 was as effective as bivalirudin and more effective than argatroban. HD1-22 also displayed slightly stronger inhibition on thrombin-induced platelet aggregation than bivalirudin and was obviously more effective than argatroban. So HD1-22 may be an ideal anticoagulant candidate when effective anticoagulation and rapid reversal of anticoagulant effect are required [27].

Heparin is the only approved anticoagulant for coronary artery bypass graft (CABG) surgery. However heparin-protamine treatment has a number of serious side effects. Especially, bleeding and thrombocytopenia are often asymptomatic but may be associated with life-threatening arterial or venous thromboemboli [28].The aptamer ARC183 (Archemix and Nuvelo) is another 15-nucleotide antithrombin DNA aptamer with desirable affinity for thrombin ($K_d = 2$ nM) and prothrombin ($K_d = 50$ nM) [19]. Experiments in vitro showed that ARC183 is a strong anticoagulant capable of inhibiting thrombin-catalyzed activation of fibrinogen and thrombin-induced platelet aggregation [29]. In dog and monkey models of cardiopulmonary bypass, ARC183 prolonged activated clotting time (ACT) in a dose-dependent manner and without acute toxicities [30,31]. Preliminary results of phase 1 trial showed that ARC183 could result in a rapid onset of anticoagulation and stable and dose-related anticoagulation activity and the blood coagulation function returned to normal again qucikly after the infusion was stopped. However the phase 1 trial was closed for a sub-optimal dosing profile. (http://www.hcp.com/content5788.html)

A second-generation antithrombin DNA aptamer, ARC1172 (NU172, Archemix Corp.), recognizes thrombin's exosite 1 and inhibits coagulation. NU172 is a short-acting anticoagulant. In the phase 1a and 1b clinical trials, intravenous injection of NU172 dose-dependently increased in ACT of healthy volunteers. No serious adverse events were reported [32]. A phase 2 trial of NU172 in patients undergoing off-pump CABG surgery is underway (NCT00808964). (http://clinicaltrials.gov).

Toggle 25 is an RNA aptamer that binds both human and porcine thrombin. Toggle 25 was developed by a new strategy named Toggle SELEX. It was selected by "toggling" the protein target between human and porcine thrombin during alternating rounds of selection. Toggle 25 inhibits both porcine and human' two of thrombin's most important functions: plasma clot formation and platelet activation by binding evolutionally conserved regions of a protein. Crystal structure analysis revealed Toggle 25 can recognize the thrombin's exosite 2 with a succession of adenine-arginine stacking interactions [33].

Since thrombin is involved in atherosclerotic plaque formation, injury repair and inflammation. antithrombin aptamers are promising antithrombotic drugs to prevent and treat thrombotic disorders like ACS and other cardiovascular disease [34].

Anti-vWF Aptamers

vWF is a multimeric glycoprotein mainly synthesized in endothelial cells and megakaryocytes. It is a protective carrier of factor VIII in the circulation. In the presence of the intravascular shear forces, at the site of endothelial damage or in atherosclerotic rupture, vWF may link to GP Ib-IX complex and mediate initial platelet adhesion, activation and aggregation, activation of the coagulation cascade, and coordination formation of the fibrin and platelet-rich thrombus at last. [35,36] Since vWF has a prominent role in various cardiovascular disease through vWF-induced platelet activation, GPIb-vWF axis is a desirable target for stroke prevention.

ARC1172 is a 41-mer DNA aptamer against vWF with high affinity to the vWF A1-domain and blocks vWF binding to GPIbα [37]. ARC1779 (Archemix Corporation) is a second anti-vWF aptamer derived from