Research in Pharmaceutical Sciences, April 2017; 12(2): 88-98
Received: August 2016
Accepted: November 2016

School of Pharmacy & Pharmaceutical Sciences
IsfahanUniversity of Medical Sciences

Review Article

Oligonucleotide aptamers: potential novel molecules against viral hepatitis

Mina Mirian[1,2], Hossein Khanahmad[1,*], Leila Darzi[2], Mansour Salehi[1], and Hojjat Sadeghi-Aliabadi[2]

[1]Department of Genetics and Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.
[2]Department of Pharmaceutical Chemistry and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

Abstract

Viral hepatitis, as an international public health concern, seriously affects communities and health system. In recent years, great strides have been taken for development of new potential tools against viral hepatitis. Among these efforts, a valuable strategy introduced new molecules called “aptamers”. Aptamers as potential alternatives for antibodies could be directed against any protein in infected cells and any components of viral particles. In this review, we will focus on recent advances in the diagnosis and treatment of viral hepatitis based on aptamer technology. In recent years, various types of aptamers including RNA and DNA were introduced against viral hepatitis. Some of these aptamers can be utilized for early and precise diagnosis of hepatitis infections and other group selected as therapeutic tools against viral targets. Designing diagnostic and therapeutic platforms based on aptamer technology is a promising approach in viral infections. The obtained aptamers in the recent years showed obvious potential for use as diagnostic and therapeutic tools against viral hepatitis. Although some modifications to increase the biostability and half-life of aptamers are underway, it seems these molecules will be a favorable substitute for monoclonal antibody in near future.

Keywords: Dissolution rate; Nanocrystal; Nanoparticle; Nanosuspension; Nanosizing

CONTENTS

1.INTRODUCTION

In spite of huge efforts, viral hepatitis remains as an international public health challenge compares with other major contagious diseases. In 2013, viral hepatitis became the seventh highest cause of death worldwide. It is responsible for about 1.4 million deaths annually from acute infection, cirrhosis and liver cancer (1,2).

*Corresponding author: H. Khanahmad
Tel: 0098 31 37922487, Fax: 0098 31 36688597
Email: hossein_khanahmad@yahoo.com

Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.202447

Of those mortality, about 47% are attributable to hepatitis B virus (HBV), 48 % to hepatitis C (HCV) and the remaining are considered for hepatitis A (HAV) and hepatitis E (HEV). Moreover, approximately 2.6 million people living with HIV are co-infected with HBV and 2.9 million with HCV. Globally, almost 240 million people suffer from chronic HBV infection and 130–150 million from chronic HCV infection (3,4).

The five main hepatitis viruses, including A, B, C, D and E show various features, especially in transmission modes, effect on populations, treatment options and health consequences. The expanded and accelerated growing rate of viral hepatitis accompanies with the lack of an effective cure or prevention strategy suffers patients and impel significant burden on communities (5,6). Nowadays, there is no conclusive treatment for HBV chronic infection and treatment protocols are extensively used as protective and symptomatic therapy, whereas, these general drugs are not capable of controlling disease efficiently. Accordingly, all activities for the prevention, treatment, and carrier detection are valuable and would be regarded as an impressive procedure to prevent the spread of virus infection (7). In recent years to resolve these concerns great strides have been taken to the development of new and potential tools against viral hepatitis. Among these efforts, one of the valuable strategies in the field of molecular medicine was introducing new molecules called “aptamers” (7-9).

“Aptamers” are single strand nucleic acid, including DNA or RNA molecules that bind to the wide range of targets from small molecules to various proteins and even cells. The term aptamer refers to the Greek aptus, i.e. “to fit”, and merus, i.e. “particle” (10).

Aptamers are recognized with high affinity and specificity to target molecule and capable of performing a particular function by folding into a specific three-dimensional structure (11). In addition to their recognition and discrimination potency, aptamers present important advantages over antibodies as they can be manipulated entirely. High stability, easy synthesis and modification, low immunogenicity and variety of targets cause aptamers reputed as chemical antibodies (12,13).

Aptamers selected through systematic evolution of ligands by exponential enrichment (SELEX) that described independently by the laboratories of Gold and Szostak in 1990. This well-developed method consists of repetitious cycles of selection and amplification during which, selected oligonucleotides with high affinity for an intended target are retained and enriched, leads to their enrichment in the pool which is finally sequenced to characterize the aptamers (14,15).

In recent years, aptamers have been introduced for various applications such as cell detection, *in vitro* and *in vivo* diagnosis, imaging, drug delivery, and biomarker discovery (16,17). They have also been widely selected to exploit as antiviral agents for detection, prevention, elimination of viral hepatitis and other pathogenic agents (18).

Aptamers were discovered more than two decades ago whoes utilization in viral diseases are not sufficiently expanded until now; although in recent years, scientists have been focusing on the application of aptamers against well-known viruses, such as human immunodeficiency virus 1 (HIV-1), HBV, HCV, human papilloma virus (HPV), severe acute respiratory syndrome (SARS) and influenza (7,19).

Among five types of viral hepatitis particular attentions have been focused on hepatitis B and C which are the leading cause of chronic hepatitis. In this review, current advances in aptamer technology against HBV and HCV infections, which are the most common and serious types of viral hepatitis will be highlighted.

2. Aptamer in the diagnosis of viral hepatitis

Early and exact diagnosis of viral hepatitis infection is a critical step for effective treatment and care. Patients with acute viral hepatitis infection present some nonspecific signs and symptoms (20). On the other hands, only less than 5% of patients with chronic hepatitis caused by viruses are aware of their status (3). In this situation, designing available

diagnostic methods can prevent further prevalence of hepatitis infection significantly. The chronic hepatitis infections are the most frequent cause of death through cirrhosis and hepatocellular carcinoma (21-23). Some useful aptasensors hve also recently been verified to detect viral hepatitis in clinic. These novel and more cost-effective diagnostic techniques, enabling early detection of HBV, are points that are very essential (24). Some diagnostic aptamers are illustrated in Table 1.

2.1. Aptamers and aptamer-based biosensors for diagnosis of HBV

The first reported aptamer for HBV diagnosis was introduced in 2010 by Liu, *et al*. The selected RNA aptamer is bound specifically to the infected hepatoma cell line which expresses hepatitis B surface antigen (HBsAg). Accordingly, this aptamer can find HBV infected cells, while in previous test only purified antigen was used as detection tool (25). After finding this aptamer Liu, *et al*. exploited fluoresce in isothiocyanate (FITC) -conjugated RNA aptamer, HBs-A22, to detect HBsAg that is expressed on the surface of infected HepG2.2.15 cells in fluorescence microscopy. Replacing FITC with pharmaceutical agents in the structure of designed RNA aptamers was proposed by this group (25). Zhijiang Xi, *et al.* selected specific single strand DNA (ssDNA) aptamers by immobilizing HBsAg on the surface of carboxylated magnetic nanoparticles (MNPs) in a SELEX process after 13 rounds of selection. Three different aptamers with a same hairpin loop structure were successfully separated *in vitro* and a chemiluminescence aptasensor based on magnetic separation and immunoassay was constructed to indicate HBsAg in pure protein form or actual serum samples. As results, the H01 aptasensor worked well and indicated high specificity for contributing to better detection of hepatitis B virus infection. The detection limit of H01 aptasensor was 0.1 ng/mL, which is five times lower than ELISA as a routine method (26). While examinations proved this construct successfully bound to the S form of HBsAg, it seems that further investigations are required to identify the aptamer cross-reactivity with other forms of HBs Ag (M and L) and the kind of interaction with particle assembly.

2.2. Aptamers and aptamer-based biosensors for diagnosis of HCV

Aptamers not only can fit into clefts on protein surfaces, particularly into the active site of enzymes to inhibit the catalytic activity like traditional antiviral small molecules, e.g., HCV protease inhibitors (boceprevir), but also they can bind to protruding parts of proteins. For this reason discovery of novel methods relying on aptamers for rapid and more cost-effective diagnosis of HCV is not unexpected.

In this regard, Lee, *et al.* obtained the construction of a biosensor utilizing the fluorescent dye (Cyanine3) RNA aptamer directed against HCV core antigen via Chip-based detection method (27), whereas Chen, *et al*. reported the ssDNA aptamer as molecular probes that bound viral glycoprotein E2 (28). The aptamers against HCV glycoprotein E2 was selected by Park, *et al*. in 2013 which used in enzyme linked apto-sorbent assay (ELASA) an innovative detection assay-provided qualitative and quantitative analysis of virus particles in the tested samples at the same time. Additionally ELASA could be a proper method for evaluation of antiviral treatment (29).

Table 1. List of aptamers and aptamer-based biosensors in viral hepatitis diagnostics.

Virus	Aptamer name	Type	Target	SELEX method	Binding affinity (Kd)	Aptamer application method	Refs.
HBV	HBs-A22	RNA	HBsAg	Protein based	n/d	Fluorescence microscopy	(25)
	H01	DNA	HBsAg	MNPs based	n/d	Chemiluminesence	(26)
HCV	9-14 9-15	RNA	Core antigen	Protein based	142 nM 224 nM	Chip-based detection	(27)
	ZE2	DNA	Glycoprotein E2	Cell based	1.05 nM	Sandwich ELISA	(28)
	E2-B E2-D	DNA	Glycoprotein E2	Protein based	4 nM 0.8 nM	ELASA	(29)

n/d, no data; MNPs, magnetic nanoparticles; ELASA, enzyme linked apto-sorbent assay.

These findings might be important for the early detection of hepatitis C while patients are in "window period", because serum antibodies have not been shown viral infection yet. Ordinarily, the fluorescent dye-conjugated aptamers seem to be valuable in various types of diagnostic assays.

3. Aptamers in the treatment of viral hepatitis

The main reason for lacking efficient vaccines or medications for treatment of viral hepatitis includes hypermutations and escaping of virouses from the host immune response (30-34). In addition, many of the current antiviral medicines cause adverse side effects and may lead to the progress of other diseases than the initial treatment. They also may weaken or enhance their actual activity due to interaction with a variety of drugs (35,36). Viral hepatitis treatment face with big drawbacks and many treatment methods have been moderately effective (37). For example, while about 0.5 million patients die every year from HCV infection, the standard treatment of HCV with ribavirin and interferon-alpha shows 50% efficiency in the infected cases (38,39). These obstacles persuade researchers to seek new alternative therapeutic tools which will be more effective and less dangerous for patients.

Aptamers as chemical antibody could be directed as potential promising tools against any protein in the infected cells and any components of viral particles (19,40). Many researches established that the best therapeutic strategy is blocking of virus penetration routes into the host cells and/or inhibition of replication enzymes (41,42). On the other hands, it is confirmed that the immune system can be selectively triggered by aptamers (43). The aptamers could be modified to improve *in vivo* biostability and coupled with a variety of secondary therapeutic agents, including small interfering RNA (siRNA), microRNA (miRNAs) and ribozymes (44,45).

According to the high prevalence of HBV and HCV infectious disease, inadequacy of antiviral drugs and well-known therapeutic problems, special attention has been recently paid to the discovery of aptamer against diverse viral targets. Some therapeutic aptamers are listed in Table 2.

Table 2. List of aptamers and aptamer-based biosensors in viral hepatitis diagnostics.

Virus	Aptamer name	Type	Target	Binding affinity (Kd)	Aptamer therapeutic effect	Refs.
HBV	S9	RNA	P protein	n/d	Reduction of replicative intermediates by about 80%–85%	(50)
	Apt.No.28	DNA	Core protein	n/d	Suppressing HBV replication in the early stages	(51)
	AO-01	DNA	MBD	180 nM	Inhibition of virion formation in cell culture	(52)
HCV	ZE2	DNA	Glycoprotein E2	1.05 nM	Blocking of viral fusion in Huh7.5.1 *cell line*	(28)
	B.2	RNA	*NS5B*	1.5 nM	Inhibition of NS5B polymerase activity	(56)
	ODN 27v	DNA	*NSB5*	132.2 nM	Reduction in virus mRNA levels	(41)
	NEO-35-s41 G925-s50	RNA	*NS3*	n/d	Inhibition of NS3 helicase and protease activity	(58)
	NEO-III 14U	RNA	*NS3*	4 nM	Protease activity inhibited in 60%	(59)
	NS5A-4 NS5A-5	DNA	NS5A	n/d	Blocking the production of new infectious virion	(60)
	NS2-1 NS2-2 NS2-3	DNA	NS2	n/d	Inhibition of virus replication and assembly	(61)
	AP30	RNA	IRES domain I	36 nM	Genetic material replication inhibited by 50%	(62)
	1-17 2-02	RNA	IRES domain II	28 nM 30 nM	Inhibition of IRES-dependent translation	(64)
	3-07	RNA	IRES domain IIId	9.6 nM	Inhibition of translation by binding to an apical loop of domain IIId	(65)
	2-02 3-07	RNA	IRES domain II and III-IV	11 nM 9.6 nM	Inhibition of IRES-dependent translation	(66)
	HH363-24	RNA	IRES domain IIId	12.28 nM	Inhibition of IRES-dependent translation	(44)

n/d, no data; MBD, matrix binding domain; *NS5B, non-structural protein 5B*; *NS3*, nonstructural protein 3; NS5A, nonstructural protein 5A; IRES, internal ribosome entry site.

3.1. Aptamers for treatment of hepatitis B virus

Some protective and symptomatic therapy such as interferon-α and nucleos(t)ide analogues (adefovir, lamivudine and entecavir), are usually prescribed for the treatment of chronic hepatitis B infection (46). Although, these medicines have therapeutic effects, their use are restricted by the severe side effects and development of drug-resistant strain (47,48).

HBV genome contains a partially relaxed circular double-stranded DNA (rcDNA), meaning that consists of a complete (−) strand and the (+) strand DNA which is variable and shorter in length (49). During hepatitis progression, this unusual DNA forms covalently closed circular DNA in the nucleus to activate RNA transcription of HBV. Viral genome translates into the four overlapping open reading frames, including core proteins (C), viral reverse DNA polymerase (P), surface antigen (S) and X protein (HBx) (49). Several target proteins encoded by HBV have been suitably subjected to the selection of aptamers.

Interaction between the viral protein R (RNA-dependent RNA polymerase) with stem-loop structure of ε sequence located at the 5' side of the pregenomic RNA is a critical step for replication and assembly of HBV. Based on this fact, Feng, *et al.* developed S9 RNA aptamer as anti-HBV replication, which interacted with viral polymerase with high affinity and competed a binding site of viral genetic material. Inhibition of replication in HBV infected cell line, HepG2.2.15, which was determined after transfection with a plasmid vector encoding S9 RNA aptamer by southern blot analysis notably showed no signs of cell toxicity in S9 aptamer-transfected cells (50).

Zhang, *et al*. in 2014 found aptamers against the core protein of HBV (HBc) from a random DNA pool by SELEX procedure. This new aptamer, Apt.No.28, demonstrated a high affinity and inhibited the assembly of the nucleocapsid, reducing extracellular DNA, whose synthesis based on the formation of the nucleocapsid, confirmed its role in suppressing HBV replication. This aptamer was suggested as a new targeting molecule to facilitate the strategy for targeted therapy of HBV-related diseases. There was no documented report for estimation of Kd (dissociation constant) for selected aptamer in this research (51).

Aptamer against matrix binding domain (MBD) on the HBV capsid surface were designed by Orabi, *et al*. After thirteen rounds of SELEX using the wild type (WT) capsids for positive selection and the I126A mutant capsids for counter selection AO-01 aptamer with the lowest Kd was selected against WT capsids. Its Kd value against the I126A mutant capsids was at least 7 fold higher. Inhibition potential test in transiently cotransfected HuH-7 cell performed 47 % HBV inhibition via AO-01 and no inhibition with an aptamer with random sequence AO-N. These results showed that the selected aptamer is specific for the MBD (52).

3.2. Aptamers for treatment of hepatitis C virus

Entry inhibitors are the most commonly studied therapeutic tools which prevent viral fusion with the target cell and penetration. Virus penetration occurs with specific surface proteins into the cells, which present as ligands.

As generally proven, many viruses such as HCV, HBV and HIV-1 have an obvious tropism for specific cell types (34).

HCV encodes two envelope glycoproteins, E1 and E2, which are released by host signal peptidase from the polyprotein precursor (53). In HCV infection, E2 glycoprotein was an important target for aptamer selection. This glycoprotein is a co-receptor of human CD81, displayed on hepatocytes and B lymphocytes (54). Chen, *et al.* obtained DNA aptamer, defined as ZE2, competitively blocking E2 in most HCV serotypes. Its efficiency was verified in Huh7.5.1, a well differentiated hepatocyte derived carcinoma cell line. The reduction in HCV RNA levels and E2 protein concentrations after treatment were demonstrated by qRT-PCR analysis and in the western blot assay respectively (28).

HCV non-structural protein 5B (NS5B) is an RNA-dependent RNA polymerase that is a desired target for aptamer therapeutics, because of its importance for the virus replication (55). Biroccio, *et al*. constructed RNA aptamer, assigned as B.2 specified by stem-loop structure, with a unique sequence UAUGGACCAGUGGC that identifies an important element -a GTP binding site of NS5B- responsible for its function. *In vitro* analysis of the polymerase activity was positively illustrated the correlation between aptamer and inhibition of polymerase activity in a concentration-dependent manner (56). Bellecave, *et al*. selected 27v as DNA aptamer against same enzyme that suppressed NS5B activity with competition the polymerase-binding site with the viral RNA template. The observation was significantly proved the viral copy number reduction in Huh7 cell line (differentiated hepatocyte derived carcinoma cell line infected with HCV JFH1 strain) by qRT-PCR which positively correlated with obtained aptamer. RNA level of virus have been declined after treatment with concentration of 5 μM, 1 μM, and 100 nM by 90%, 68%, and 19%, respectively comparing with non-aptamer treated cells (7.8×10^6 to 22×10^6 HCV RNA copies). The aptamer molecules were observed intracellularly using confocal microscopy, despite the absence of the transfection agent (41).

Among other enzymes, which indirectly play role in virus replication, nonstructural protein 3 (NS3) showed helicase and protease activity which is related to the C- and N-terminal domains of the enzyme, respectively. For the replication of the flaviviridae family, including HCV, both domains of NS3 are essential. Protease domain changes viral proteins necessary for its life cycle, while helicase unwinds DNA and RNA duplexes and then unpackaged genetic material replicates by polymerase. The helicase domain of HCV NS3 binds preferentially to the poly (U) sequence situated in the 3'-untranslated region of the viral genome (3'-UTR) under normal conditions (57). Umehara, *et al*. created a bivalent aptamer with sequences coupled with a poly (U) linker. In this study, they determined the optimum length of the linker, i.e., 41 and 50 nucleotides. Consequently, NEO-35-s41 and G925-S50 aptamers showed highest synchronous reduction in both helicase and protease activity of NS3. These results were confirmed using an *in vitro* enzymatic assay (58).

In another study, Fukuda, *et al*. introduced ΔNEO-III-14U RNA aptamer, which powered by a competitor sequence (poly U), as characterized during *in vitro* enzymatic and *in vivo* tests. Selected aptamer significantly inhibited the activity of both NS3 protease and helicase domains in a dose-dependent manner. They supposed that ΔNEO-III-14U aptamer can compete with HCV in the 3'-UTR regions of genome for the binding site of helicase domain (59).

Nonstructural protein 5A (NS5A) is a zinc-binding and proline-rich hydrophilic phosphoprotein that plays a critical role in RNA replication of HCV and its assembly. Yu, *et al*. developed NS5A-4 and NS5A-5 aptamers, demonstrated one-fold decrease in viral RNA level after anti-NS5A aptamers treatment in Huh7.5 cells compared with control, as estimated by real-time PCR. The results of focus forming assay on naive Huh7.5 cells indicated coupling of NS5A by the aptamer that could block the production of new infectious virion. It seems when interferon response to viral infection remains inactive, this viewpoint-specific is efficient (transcription of interferon genes was evaluated by qRT-PCR) (60). Gao, *et al*. with accordant results about the safety approach established NS2-1, NS2-2 and NS2-3 aptamers against NS2 protein (nonstructural protein 2) of the HCV, which can powerfully influence on viral replication (61).

As an opportunity, aptamers not only can recognize viral proteins, but also able to detect its nucleic acids. Designing aptamers for specified region of HCV genome, which interact with viral life cycle involve proteins appears to be an impressive therapeutic tool. The internal ribosome entry site (IRES) sequence, associated with viral translation, is well-conserved among HCV isolates because it is a potential attractive target for anti-HCV drugs. IRES consists of four domains (I-IV) present in the 5'-untranslated region

(5'-UTR) of the HCV genome, which allow cap and end-independent mRNA translation in the host cell. The internal initiation of translation begins with the binding of IRES to the small ribosomal subunit (40S) in the host cell, and eukaryotic translation initiation factor 3 (eTIF3). In 2011 Konno, *et al*. rendered RNA aptamer AP30 that targeted domain I of IRES in 3' end of the genome antisense strand. AP30 showed approximately 50% replication suppression via *in vitro* analysis. Two consensus sequences 5'-UGGAUC-3' and 5'-GAGUAC-3', which completely bound to the SL-E1 and SL-D1 loops in the domain I performed attachment prevention of NS5B (62,63). Kikuchi, *et al*. applied RNA aptamer containing loop structure including a consensus sequence 5'-UAUGGCU-3', complementary to the loop of the IRES domain II. About 20%–40% reduction in luciferase activity was established by *in vitro* translation test of IRES-luciferase mRNA due to the exposure of 1-17 and 2-02 aptamers (64). In another study, the same team of researchers isolated aptamer 3-07 capable of binding to the IIId domain of IRES. This aptamer has positively operated by viral *in vitro* IRES-dependent translation blockage and targeted the second domain of IRES. A bout 10% depletion in the luciferase activity compared to the control levels was detected. The potency and selectivity of this aptamer was shown in transfected HeLa cells with 0.5 pmol 3-07 aptamer which demonstrated inhibition in luciferase activity up to 45% (65). Especial attention was paid to synchronous inhibition of IRES domains II and III-IV; particularly, IIId and IIIe regions as essential elements in viral translation. Consequently, conjugated forms of two aptamers, named as 2-02 and 3-07, was produced and proved a 10 times decrease in binding affinity to the target sequence than the two parental aptamers; also the IC50 value showed same decrease in translational activity, 10-fold lower than components alone (66). In addition, Romero-Lopez, *et al*. generated HH363-24 molecule that was an engineered aptamer composed the activity of hammer head ribozyme (HH363) with properties typical for aptamers. This construct bound to the essential IIId domain of the IRES, cleaved the HCV genome in 3' side and inhibited viral translation and replication simultaneously. HH363-24 have proved to be highly potent inhibitory molecules in Huh7.5.1 cells, containing HCV-1b subgenomic RNA replicons. HH363-24 also was efficiently inhibited HCV RNA synthesis up to 70% in a subgenomic replicon system in the qRT-PCR assay. Mutational analysis exposed that combining aptamers for various target recognition sites improved the inhibition activity by increasing the domain binding competency (44).

4. Challenges of commercialization

In the recent years smart aptamers with ability of binding to a wide range of targets, from ions, small molecules to large macromolecules such as proteins and viruses were released. Identification of various targets would be valuable, especially when there is no prior knowledge about target molecules that frequently occur with virus mutations for evasion of the host immune system. Accordingly, aptamer selection can be implemented without exact target information and then *in vitro* and *in vivo* validation would be carried out for evaluation of its efficacy. Aptamers additionally possess several key advantages in comparison to antibodies such as thermal stability, low immunogenicity, easily scalable and cost-effective. Beside all of the brilliant advantages of aptamers, commercialization process faces with some barriers affecting therapeutic potency *in vivo* (67).

4.1. Aptamer degradation under physiological conditions

One of the main challenges of aptamer commercialization is serum endo- or exonucleases protection. Nowadays, based on nucleases sensitivity of unmodified nucleotides various chemical modifications can be used into the nucleotide sugars or internucleotide phosphodiester linkages. These modifications decrease obviously aptamer degradation rate, which occur with *in vivo* nucleases. Furthermore, natural form of nucleic acids can be replaced with synthetic

nucleic acids like Xeno nucleic acid (XNA). These unnatural polymers not only will mimic DNA and RNA function, but demonstrate significant enhancement of nuclease resistance and then display longer half-lives *in vivo* (68,69).

4.2. Renal filtration

Although rapid clearance in aptamer approach would be helpful for *in vivo* diagnostic imaging, but it can influence destructively on other biomedical applications. The renal filtration threshold is thought to be 30-50 kD usually. Antibodies due to their large size (~150 kD) and special recycling system circulate in the bloodstream with extended half-life, while clearance of non-modified aptamer happen quickly from the blood, with a half-life of minutes to hours. However, some tricks such aptamer conjugation via high molecular weight polymers like polyethylene glycol (PEG) not only can significantly enhance its half-life under physiological conditions but also improve stability and reduce aptamer toxicity accumulation in nontarget organs (70).

4.3. Toxicity

Aptamers as therapeutic agents can reveal toxicity effects in both on-target and off-target situations. Although clinical trials of the limited number of aptamers are being conducted, but studies generally indicated moderate or low toxicity effects of aptamers.

Other investigations have represented some oligonucleotide conjugation partners such as PEG or other high-molecular weight compounds may stimulate the production of antibodies that neutralize aptamers in bloodstream and reduce toxicity (71,72).

4.4. Intellectual property

The therapeutic potentials and applications of aptamer may be limited by intellectual property rights. Now aptamers and their selection technologies are widely supported by a single intellectual property portfolio and therefore investment in this area is depend on the collaboration between researchers and companies. In contrast, antibody technologies are currently applied broadly for lack of patent protection or expiration of intellectual property rights. Although these protection rights for the SELEX method will expire in the near future (68).

5. CONCLUSION

As outlined in this review, recent advances in aptamer technology, specifically recognizing viruses disclosed significant potential in a range of diagnostic and therapeutic applications. These molecules as a convincing substitute for antibodies have provided an effective tool for viral infection management.

Designing diagnostic platforms based on aptamer technology is a promising approach in viral infections. Aptamers with special features such as detection of early or late viral markers, ability to discriminate infected cell from normal or active and inactive virus looks extremely hopeful. Despite the obstacles of *in vivo* studies, several aptamers are undergoing clinical trials. Therapeutic aptamers can be effectively designed and applied against viral hepatitis by various mechanisms including blockage of virion penetration, inhibition of viral replication, delivery of antiviral agents and activation of immune system. Accordingly, aptamers can operate diverse commercial applications to combat viral hepatitis by applying a series of modifications in pharmacokinetic properties.

REFERENCES

1. Bosetti C, Turati F, La Vecchia C. Hepatocellular carcinoma epidemiology. Best Pract Res Clin Gastroenterol. 2014;28:753-770.
2. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57:1333-1342.
3. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. The Lancet. 2015;386:1546-1555.
4. Zary N, MohammadReza Y, Majid Y, Parisa S, Mina M, Anahita B, *et al*. Prevalence and risk factors of HIV, syphilis, hepatitis B and C among female prisoners in Isfahan, Iran. Hepat Mon. 2012;2012:442-447.

5. Cooke G, Lemoine M, Thursz M, Gore C, Swan T, Kamarulzaman A, et al. Viral hepatitis and the Global Burden of Disease: a need to regroup. J Viral Hepat. 2013;20(9):600-601.
6. Hope V, Eramova I, Capurro D, Donoghoe M. Prevalence and estimation of hepatitis B and C infections in the WHO European Region: a review of data focusing on the countries outside the European Union and the European Free Trade Association. Epidemiol Infec. 2014;142(2):270-286.
7. Mirian M, Taghizadeh R, Khanahmad H, Salehi M, Jahanian-Najafabadi A, Sadeghi-Aliabadi H, Kouhpayeh Sh. Exposition of hepatitis B surface antigen (HBsAg) on the surface of HEK293T cell and evaluation of its expression. Res Pharm Sci. 2016;11:366-373.
8. Binning JM, Leung DW, Amarasinghe G. Aptamers in virology: recent advances and challenges. Front Microbiol. 2012;3:29.
9. Shum K-T, Zhou J, Rossi JJ. Aptamer-based therapeutics: new approaches to combat human viral diseases. Pharmaceuticals. 2013;6:1507-1542.
10. Famulok M, Mayer Gn. Aptamer modules as sensors and detectors. Acc Chem Res. 2011;44:1349-1358.
11. Kadioglu O, Malczyk AH, Greten HJ, Efferth T. Aptamers as a novel tool for diagnostics and therapy. Invest New Drugs. 2015;33:513-520.
12. Vallian S, Khazaei M. Medical applications of aptamers. Res Pharm Sci. 2009;2:59-66.
13. Kouhpayeh S, Einizadeh A, Hejazi Z, Boshtam M, Shariati L, Mirian M, *et al*. Antiproliferative effect of a synthetic aptamer mimicking androgen response elements in the LNCaP cell line. Cancer Gene Ther. 2016. 23(8):254-257.
14. Sefah K, Shangguan D, Xiong X, O'Donoghue MB, Tan W. Development of DNA aptamers using Cell-SELEX. Nat Protoc. 2010;5:1169-1185.
15. Ohuchi S. Cell-SELEX technology. Biores Open Access.. 2012;1:265-272.
16. Ye M, Hu J, Peng M, Liu J, Liu J, Liu H, *et al*. Generating aptamers by cell-SELEX for applications in molecular medicine. Int J Mol Sci. 2012;13:3341-3353.
17. Song K-M, Lee S, Ban C. Aptamers and their biological applications. Sensors. 2012;12:612-631.
18. Mescalchin A, Restle T. Oligomeric nucleic acids as antivirals. Molecules. 2011;16:1271-1296.
19. Wandtke T, Woźniak J, Kopiński P. Aptamers in diagnostics and treatment of viral infections. Viruses. 2015;7:751-780.
20. Aspinall E, Hawkins G, Fraser A, Hutchinson S, Goldberg D. Hepatitis B prevention, diagnosis, treatment and care: a review. Occup Med.2011;61:531-540.
21. Raffetti E, Fattovich G, Donato F. Incidence of hepatocellular carcinoma in untreated subjects with chronic hepatitis B: a systematic review and meta-analysis. Liver Int. 2016. 36(9):1239-1251
22. Papatheodoridis GV, Chan HL-Y, Hansen BE, Janssen HL, Lampertico P. Risk of hepatocellular carcinoma in chronic hepatitis B: assessment and modification with current antiviral therapy. J Hepatol. 2015;62:956-967.
23. Sinn DH, Lee J, Goo J, Kim K, Gwak GY, Paik YH, *et al*. Hepatocellular carcinoma risk in chronic hepatitis B virus–infected compensated cirrhosis patients with low viral load. Hepatology. 2015;62:694-701.
24. Singh R, Mukherjee MD, Sumana G, Gupta RK, Sood S, Malhotra B. Biosensors for pathogen detection: A smart approach towards clinical diagnosis. Sens Actuators B Chem. 2014;197:385-404.
25. Liu J, Yang Y, Hu B, Ma Z-y, Huang H-p, Yu Y, *et al.* Development of HBsAg-binding aptamers that bind HepG2. 2.15 cells via HBV surface antigen. Virologica Sinica. 2010;25:27-35.
26. Xi Z, Huang R, Li Z, He N, Wang T, Su E, *et al*. Selection of HBsAg-specific DNA aptamers based on carboxylated magnetic nanoparticles and their application in the rapid and simple detection of hepatitis B virus infection. ACS Appl Mater Interfaces. 2015;7:11215-11223.
27. Lee S, Kim YS, Jo M, Jin M, Lee D-k, Kim S. Chip-based detection of hepatitis C virus using RNA aptamers that specifically bind to HCV core antigen. Biochem Biophys Res Commun. 2007;358:47-52.
28. Chen F, Hu Y, Li D, Chen H, Zhang X-L. CS-SELEX generates high-affinity ssDNA aptamers as molecular probes for hepatitis C virus envelope glycoprotein E2. PLoS One. 2009;4:e8142.
29. Park JH, Jee MH, Kwon OS, Keum SJ, Jang SK. Infectivity of hepatitis C virus correlates with the amount of envelope protein E2: development of a new aptamer-based assay system suitable for measuring the infectious titer of HCV. Virology. 2013;439:13-22.
30. Lazarevic I. Clinical implications of hepatitis B virus mutations: recent advances. World J Gastroenterol. 2014;20:7653-7664.
31. Marascio N, Torti C, Liberto MC, Focà A. Update on different aspects of HCV variability: focus on NS5B polymerase.BMC Infect Dis. 2014;14(1):148.
32. Finlay BB, McFadden G. Anti-immunology: evasion of the host immune system by bacterial and viral pathogens. Cell. 2006;124:767-782.
33. Kramvis A. Genotypes and genetic variability of hepatitis B virus. Intervirology. 2014;57:141-150.
34. Cuypers L, Li G, Libin P, Piampongsant S, Vandamme A-M, Theys K. Genetic diversity and selective pressure in hepatitis c virus genotypes 1–6: significance for direct-acting antiviral treatment and drug resistance. Viruses. 2015;7:5018-5039.
35. Soriano V, Labarga P, Barreiro P, Fernandez-Montero JV, de Mendoza C, Esposito I, *et al*. Drug interactions with new hepatitis C oral drugs. Expert Opin Drug Metab Toxicol. 2015;11:333-341.
36. Zornitzki T, Malnick S, Lysyy L, Knobler H. Interferon therapy in hepatitis C leading to chronic type 1 diabetes. World J Gastroenterol. 2015;21:233-239.
37. Munir S, Saleem S, Idrees M, Tariq A, Butt S, Rauff B, *et al*. Hepatitis C treatment: current and future perspectives. Virol J. 2010;7:296.

38. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, *et al*. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. The Lancet. 2001;358:958-965.
39. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, *et al*. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the global burden of disease study 2010. The Lancet. 2013;380:2095-2128.
40. Ni X, Castanares M, Mukherjee A, Lupold SE. Nucleic acid aptamers: clinical applications and promising new horizons.Curr Med Chem. 2011;18:4206-4214.
41. Bellecave P, Cazenave C, Rumi J, Staedel C, Cosnefroy O, Andreola M-L, *et al*. Inhibition of hepatitis C virus (HCV) RNA polymerase by DNA aptamers: mechanism of inhibition of in vitro RNA synthesis and effect on HCV-infected cells. Antimicrob Agents Chemother. 2008;52:2097-2110.
42. Yang D, Meng X, Yu Q, Xu L, Long Y, Liu B, *et al*. Inhibition of hepatitis C virus infection by DNA aptamer against envelope protein. Antimicrob Agents Chemother.. 2013;57:4937-4944.
43. Hwang S-Y, Sun H-Y, Lee K-H, Oh B-H, Cha YJ, Kim BH, *et al*. 5′-Triphosphate-RNA-independent activation of RIG-I via RNA aptamer with enhanced antiviral activity. Nucleic Acids Res. 2012;40:2724-2733.
44. Romero-Lopez C, Berzal-Herranz B, Gómez J, Berzal-Herranz A. An engineered inhibitor RNA that efficiently interferes with hepatitis C virus translation and replication. Antiviral Res. 2012;94:131-138.
45. Zhu Q, Shibata T, Kabashima T, Kai M. Inhibition of HIV-1 protease expression in T cells owing to DNA aptamer-mediated specific delivery of siRNA. Eur J Med Chem. 2012;56:396-399.
46. Caviglia G, Abate M, Pellicano R, Smedile A. Chronic hepatitis B therapy: available drugs and treatment guidelines. Minerva Gastroenterol Dietol. 2015;61:61-70.
47. Khungar V, Han S-H. A systematic review of side effects of nucleoside and nucleotide drugs used for treatment of chronic hepatitis B. Curr Hepat Rep. 2010;9:75-90.
48. Negro F. Adverse effects of drugs in the treatment of viral hepatitis. Best Pract Res Clin Gastroenterol. 2010;24:183-192.
49. Dandri M, Locarnini S. New insight in the pathobiology of hepatitis B virus infection. Gut. 2012;61:6-17.
50. Feng H, Beck J, Nassal M, Hu K-h. A SELEX-screened aptamer of human hepatitis B virus RNA encapsidation signal suppresses viral replication. PLoS One. 2011;6:e27862.
51. Zhang Z, Zhang J, Pei X, Zhang Q, Lu B, Zhang X, *et al*. An aptamer targets HBV core protein and suppresses HBV replication in HepG2. 2.15 cells. Int J Mol Med. 2014;34:1423-1429.
52. Orabi A, Bieringer M, Geerlof A, Bruss V. An aptamer against the matrix binding domain on the hepatitis B virus capsid impairs virion formation. J Virol.2015;89:9281-9287.
53. Bartenschlager R, Penin F, Lohmann V, André P. Assembly of infectious hepatitis C virus particles. Trends Microbiol. 2011;19:95-103.
54. Zeisel MB, Fofana I, Fafi-Kremer S, Baumert TF. Hepatitis C virus entry into hepatocytes: molecular mechanisms and targets for antiviral therapies. J Hepatol. 2011;54:566-576.
55. Lohmann V. Hepatitis C virus RNA replication. Hepatitis C virus: from molecular virology to antiviral therapy: Springer; 2013;369:167-198.
56. Biroccio A, Hamm J, Incitti I, De Francesco R, Tomei L. Selection of RNA aptamers that are specific and high-affinity ligands of the hepatitis C virus RNA-dependent RNA polymerase. J Virol. 2002;76:3688-3696.
57. Kanai A, Tanabe K, Kohara M. Poly (U) binding activity of hepatitis C virus NS3 protein, a putative RNA helicase. FEBS letters. 1995;376:221-224.
58. Umehara T, Fukuda K, Nishikawa F, Sekiya S, Kohara M, Hasegawa T, *et al*., editors. Designing and analysis of a potent bi-functional aptamers that inhibit protease and helicase activities of HCV NS3. Nucleic acids symposium series. Oxford Univ Press. 2004;48:195-196.
59. Fukuda K, Umehara T, Sekiya S, Kunio K, Hasegawa T, Nishikawa S. An RNA ligand inhibits hepatitis C virus NS3 protease and helicase activities. Biochem Biophys Res Commun. 2004;325:670-675.
60. Yu X, Gao Y, Xue B, Wang X, Yang D, Qin Y, *et al*. Inhibition of hepatitis C virus infection by NS5A-specific aptamer. Antiviral Res. 2014;106:116-124.
61. Gao Y, Yu X, Xue B, Zhou F, Wang X, Yang D, *et al*. Inhibition of hepatitis C virus infection by DNA aptamer against NS2 protein. PLoS One. 2014;9:e90333.
62. Konno K, Fujita S, Iizuka M, Nishikawa S, Hasegawa T, Fukuda K, editors. Isolation and characterization of RNA aptamers specific for the HCV minus-IRES domain I. Nucleic acids symposium series. Oxford Univ Press. 2008;52:493-494.
63. Konno K, Iizuka M, Fujita S, Nishikawa S, Hasegawa T, Fukuda K. An RNA aptamer containing two binding sites against the HCV minus-IRES domain I. Nucleosides, Nucleotides and Nucleic Acids. 2011;30:185-202.
64. Kikuchi K, Umehara T, Fukuda K, Hwang J, Kuno A, Hasegawa T, *et al*. RNA aptamers targeted to domain II of hepatitis C virus IRES that bind to its apical loop region. J Biochem. 2003;133:263-270.
65. Kikuchi K, Umehara T, Fukuda K, Kuno A, Hasegawa T, Nishikawa S. A hepatitis C virus (HCV) internal ribosome entry site (IRES) domain III–IV-targeted aptamer inhibits translation by binding to an apical loop of domain IIId. Nucleic Acids Res. 2005;33:683-692.
66. Kikuchi K, Umehara T, Nishikawa F, Fukuda K, Hasegawa T, Nishikawa S. Increased inhibitory

ability of conjugated RNA aptamers against the HCV IRES. Biochem Biophys Res Commun. 2009;386:118-123.
67. Sun H, Zu Y. A highlight of recent advances in aptamer technology and its application. Molecules. 2015;20:11959-11980.
68. Keefe AD, Pai S, Ellington A. Aptamers as therapeutics. Nat Rev Drug Discov. 2010;9:537-550.
69. Bruno JG. Predicting the uncertain future of aptamer-based diagnostics and therapeutics. Molecules. 2015;20:6866-6887.
70. Lakhin A, Tarantul V, Gening L. Aptamers: problems, solutions and prospects. Acta Naturae. 2013;5:34-43.
71. Radom F, Jurek PM, Mazurek MP, Otlewski J, Jeleń F. Aptamers: molecules of great potential. Biotechnol adv. 2013;31:1260-1274.
72. Sundaram P, Kurniawan H, Byrne ME, Wower J. Therapeutic RNA aptamers in clinical trials. Eur J Pharm Sci. 2013;48:259-271.