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Can New Strategies Be An Alternative To Antiviral Drug Resistance?

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SUMMARY Viral agents have been continuing to cause life threatening chronic infections, deaths and economic losses in humans and animals. Recently a limited number of antiviral drug is available to treat human, animal and zoonotic virus infections. The emergence of resistance to these antivirals has created an obstacle for the treatment of patients who infected with viruses. To struggle with viral infections, there is need to prevent the emergence of antiviral drug resistance by developing the new strategies or tactics. Recently fidelity variants and lethal mutagenesis is considered to be an effective strategy for the prevention of drug resistance. This review summarises the new strategies and antiviral mechanisms being tried to develop in order to fight viral infections.

Key Words: *Virus, Antiviral drugs, Lethal mutagenesis, Fidelity variants*

ÖZET

Yeni Stratejiler Antiviral İlaç Direncine Alternatif Olabilir mi?

Viral ajanlar, insanlarda ve hayvanlarda hayatı tehdit eden kronik enfeksiyonlara, ölümlere ve ekonomik kayıplara neden olmaya devam etmektedir. Günümüzde insan, hayvan ve zoonotik virus enfeksiyonlarının tedavisi için sınırlı sayıda antiviral ilaç mevcuttur. Oluşan antiviral ilaç direnci bu viruslar ile enfekte hastaların tedavisi için engel teşkil etmektedir. Viral enfeksiyonlar ile mücadele için yeni stratejiler ya da taktikler geliştirerek oluşan antiviral ilaç direncinin önlenmesine ihtiyaç vardır. Günümüzde fidelite varyantlarının ve letal mutagenezin ilaç direncinin önlenmesi için etkili stratejiler olduğu düşünülmektedir. Bu derleme viral enfeksiyonlar ile mücadele amacı ile geliştirilmesi denenen yeni stratejileri ve antiviral mekanizmaları özetlemektedir.

Anahtar Kelimeler: *Virus, Antiviral ilaçlar, Letal mutagenez, Fidelite varyantları*

INTRODUCTION

As the most important pathogens to produce casualties, chronic life-long diseases or economic losses, viruses have been continuing to cause a global threat (Lazaro 2011, Lou et al. 2014, Martinez et al.2015). These agents can infect all species on Earth including human, animal, plant, insect, bacteria as well as archaea (Costa et al. 2012).At all stages of life, many of the dangers to human health arise from viral infections such as haemorrhagic fever viruses including *Ebola virüs (EBOV)*, *Crimean Congo Haemorrhagic Fever virus (CCHFV), human immunodeficiency virus (HIV)*, *SARS-coronavirus, influenza virus*, *hepatitis A, B* and C *viruses (HAV, HBV and HCV)*. Similarly, some viruses such as *bluetongue virus (BTV), foot and mouth disease virüs (FMDV)*, *pestiviruses* present the greatest risk to animals (Costa et al. 2012).In the course of history, several sizeable viral outbreaks have resulted in the large-scale deaths of animals and human beings. For instance, the influenza pandemic of 1918, described as a global disaster, killed 50 million people which is believed to be higher than the number of people who died in World War 1 (Taubenger et al. 2006). Likewise, in the 18th and 19thcenturies, *rinderpest virus* infection, known as 'cattle plague', spread all over the world with hardly a continent or country left unaffected by this virus. While 200 million cattle in Western Europe were killed by *rinderpest virus* that is known as one the most important *morbilliviruses*, 80 to 90% of all cattle population in South Africa were extinguished (Mack 1970). However, at the beginning of the 21th century, mankind was threatened by two major *influenza virus* outbreaks reminiscent of the 1918 flu pandemic. More recently still, the huge threat to human health posed by the EBOV was demonstrated by the deaths of more than 11.000 people in West Africa (WHO 2015).

Despite the availability of highly effective strategies such as vaccination, public health measures and improvement of hygiene use for the control and eradication of viral diseases in populations, only two known viruses, *smallpox virus* and *rinderpest virus*, have been officially eradicated all over the world, and there are large numbers of viral diseases which still escape control (Martinez et al.2015). In recent years, resistance to antiviral drugs is increasingly

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reported as a major challenge, due largely to their over-prescription and over-usage (Costa et al. 2012). In addition, since RNA viruses have flexible genetic structure mutating quickly, and mutations in their genes can lead to the development of the resistance to antiviral drugs, this presents a growing challenge for immunocompromised patients particularly those infected with HIV and HCV (Costa et al. 2012).

MUTATION AND MUTATION FREQUENCIES OF RNA AND DNA VIRUSES

All viruses have either DNA or an RNA genome which always stores their genetic codes. Most RNA viruses cause serious infections to humans as well as animals. Currently, none of the DNA viruses appear in the top of the most serious infectious diseases list whilst RNA viruses are reported to have taken place in the front rows (Belshaw et al. 2008). For those viruses, mutation is their most important feature. Mutational changes occur within the nucleotide sequences of virus genomes. By comparison, it appears that RNA viruses have higher mutation frequency than DNA viruses due to the absence of proof-reading activity associated with RNA- dependent-RNA-polymerases (RdRPS) which known as the enzyme responsible for replication of RNA genome (Graci et al. 2012, Meng and Kwang 2014, Rozen et al. 2014). RNA viruses have 10^{-5} mutations per incorporated nucleotides whilst DNA viruses have 10^{-8} to 10^{-11} error per base pair (Graci et al. 2012). Many RNA viruses infect their hosts which have adaptive immune response that recognize and destroy pathogens. The high mutation of RNA viruses facilitates their escape from neutralising antibodies (Meng and Kwang 2014, Zeng et al. 2014, Pauly and Laurings 2015). Furthermore it provides great adaptability for RNA viruses to restrict the effects of vaccines and antiviral treatment. However there are also negative consequences of mutations which make RNA viruses highly susceptible to those additional mutations known to have deleterious effects on viral fitness, and on the ability to adapt rapidly to new selective pressure (Lazaro 2014).

HIGH AND LOW FIDELITY VARIANTS

Fidelity of RNA viruses is an important factor to constrain within a rangewhich balances virus replication, pathogenesis and tissue tropism that is needed for virus growth (Campagnola et al. 2014, Smith et al. 2014).Viral RDRPs are known to have high mutation rates generating low fidelity mutants (known as mutator variants) as well as high fidelity mutants known as antimutator variants (Campagnola et al. 2014, Novella et al. 2014, Rozen et al. 2014, Xie et al. 2014).There are some differences amongst these mutants (Novella et al. 2014). High fidelity mutants of RNA viruses have higher genetic stability than wild type virus, replicate slowly, generate fewer RNA genomes with great accuracy and a higher specific infectivity; in contrast low fidelity mutants have a high mutation frequency with many errors, replicate quickly, synthesise more RNA genomes which have a lower specific infectivity (Meng and Kwang 2014, Novella et al. 2014, Rozen et al. 2014, Khantun et al. 2015).Unlike low fidelity mutants, high fidelity mutants have a lower mutation frequency due to RdRPs catalysing the replication of genome slowly, thus have a better chance to reject a nucleotide pair improperly (Novella et al. 2014). Despite these differences, overall growth and titers of both high and low fidelity mutants are not significantly different *in vitro* (Rozen et al. 2014. Many researchers have reported that although undergoing genetic changes, high fidelity mutants do not have replicative problems in mammalian cells *in vit*ro and may reach high titers in the relevant cells, behaving like a wild type virus. Moreover, these mutants may attenuate with failures in replication and spread *in vivo* (Lazaro 2014). Low fidelity mutants do not also have replicative problems *in vitro* (Novella et al. 2014, Rozen et al. 2014). Furthermore they are attenuated in vivo like high fidelity mutants (Novella et al. 2014, Rozen et al. 2014).The high mutation rate with low fidelity in RNA viruses is reported to be due to three main reasons including a life history, a variety of replication speed and constraintson virus evolution. Depending on these reasons, there are also three major consequences, whichinclude population viability, mutational robustness and small genome (Graci et al. 2008).Hence, these cause-result relationships affect pathogenesis and transmission of RNA viruses during natural infections (Arisa et al. 2014, Smith et al.2014).

ANTIVIRAL DRUGS and DEVELOPMENT OF DRUG-RESISTANT MUTANTS

Currently, antiviral compounds can be categorized into two groups according their effects on viruses and host, consisting of (i) virus-acting antivirals (VAAs) that directly or indirectly target the functions of viral proteins, enzymes and the stages of virus replication cycle, and (ii) host-acting antivirals (HAAs) that regulate the immune response and cellular process of a host (Lou et al. 2014, Martinez et al.2015). In recent times, VAAs are most commonly applied in the treatment of HIV, HCV and HBV, herpes and influenza viruses. In 2014, it has been reported that of the 50 known VAAs approved by the American Food and Drug Administration (FDA), 26 are used against HIV (Martinez et al.2015). In addition to, interferon, antibodies and vaccines are also known to be HAAs are applied against viral infections (Lou et al. 2014, Martinez et al.2015). Most VAAs have a direct inhibitory effect on viral proteins and enzymes which include polymerases and proteases (Lou et al. 2014).When they are applied, attachment, entry, polymerase and protease activities are inhibited; thus the titer of virus starts decreasing to such an extent that, in terms of the immune system, this might be an opportunity to clear infection. In the implementation of these drugs, it is inevitable that mutations causing single amino acid replacement can become, and result in the emergence of, drug-resistant virus mutants (DRVM) (Lazaro 2011). These mutations might not have been important when evaluating in terms of acute viral infection; because the immune system succeeds in controlling the replication of virus as well as DRVM.

Nevertheless, the emergence of DRVM is a serious problem for those persistent infections which allow sufficient time for the natural selection that causes single amino acid mutations, thus permitting the growth of resistant viruses which cause treatment failure. In presence of drugs, emerging DRVM can be low frequency in the virus population until their replication exceeds the rest of mutants (Lazaro 2011). Furthermore, it is possible to confront with compensatory mutations that can help DVRM to increase their fitness. This may create a risk for treatment because, even if it stops, resistance and transmission of DVRM may continue to be implicated in an increasingly serious problem for the treatment of persistent infections such as HIV, HCV (Lazaro 2011). In this case we are left to wonder how we will find a successful therapy for RNA viruses, or how we will protect people during outbreaks of highly pathogenic viral infections, such as those caused by influenza virus, ebola virus and others. DRVM seems to be a hurdle or an antiviral monotherapy and can be overcome by using

alternative methods such as combination antiviral therapy (CAT) via VAAs and broad-spectrum antiviral therapy (BSAT) ((Lazaro 2011, Martinez et al.2015).

CAT is known to be a successful strategy to reduce DRVM consisting of simultaneously giving the combination of several VAAs that target either treatment of infection or suppression of its symptoms (Martinez et al.2015). VAAs focus on the specific area of viruses in replication stages, and combined administration of anti-HCV protease and polymerase inhibitors for all chronic HCV treatment is the best example of this strategy (Martinez et al.2015). Likewise, highly active antiretroviral therapy (HAART) which is used for HIV treatment is another notable example. This therapy consists of the combination of one or two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase, or one protease inhibitor. The success of CAT strategies is considerable. Otherwise, there are still some ongoing unsolved issues in CAT that can particularly become in coinfections developing with main infections. These are comprised (i) the emergence of cross drug-resistance, that would reduce therapy efficiency, (ii) toxicity created by drug-drug interaction, (iii) poor treatment response, (iv) emergence of resistance against virus (Lazaro 2011, Martinez et al.2015).

LETHAL MUTAGENESIS

Researchers have demonstrated that developing drug resistance is a significant threat for future treatment of viral infections with antivirals, because the high error rates of RNA viruses provide them with great adaptability (Lazaro 2011). On the other hand, the consequences of this high error rate mean that RNA viruses are highly susceptible to mutations which cause deleterious effects on their fitness, leading to the extinction of virus populations (Arisa et al.2014). If the error threshold is crossed, the loss of virus infectivity that depends on the loss of genetic information would be inevitable (Lazaro 2011). These observations are incorporated into lethal mutagenesis (LM) proposed as a novel antiviral strategy which has recently begun to find favour amongst those looking into its clinical applications (Perales et al. 2011). The first time the term "lethal mutagenesis (LM)" was used by Loeb et al. (1999) in their article which had published the results of research on interactions between mutagenic pyrimidine analogue and HIV replication in cell culture (Perales et al. 2011). Following this research which had suggested the use of mutagenic agents anti-retroviral drugs, many studies have been performed. The important results obtained by studying of virus extinction with *in vitro* encouraged to create *in vivo* studies about LM. Firstly it has been reported that 5-Fluorouracil (5-FU), a nucleosid analoque, has positive effects on preventing persistent *lymphocytic choriomeningitis virus* (LCMV) infection in mice *in vivo* (Ruiz-Jarabo et al. 2003). Furthermore 5-FU is given to HIV patientsin clinical trials and this is seen as a first encouraging step for clinical applications of LM (Mullins et al. 2011). Basically, LM was inspired by surpassing error threshold or transition into error catastrophe. If LM is evaluated in terms of targets and purposes, it can be seen that the target is the genetic information of virus using mutations to bring about a reduction in viral fitness. As a result of mutations, a virus can lose its genetic information when crossing an error threshold. The aim is to achieve a significant decrease in virus load, to limit virus viability or bring about viral extinction by increasing mutation rate of RNA viruses. Mutagenic nucleoside analogues (NAs), recommended for treatment of various viral infections e.g *herpesvirus*, HCV, HBV and CCHFV, are incorporated into viral RNA genomes during RNA synthesis, resulting in a significant increase in the frequency of deleterious mutations of RNA viruses (Baskin et al. 2005, Bull et al. 2007, Igde and Yazici 2012, Khantun et al. 2015, Pauly and Laurings 2015). These compounds can cause a virus to cross an error threshold thought to be brought about by LM which exploits the high mutational rate and low mutational tolerance of many RNA viruses (Pauly and Laurings 2015). Depending on the consequence of increasing mutation rate, a virus escape would be significantly lessened by these compounds. Mutagenic NAs must have some particular characteristics, including the need not to be toxic for cells, to be specific for viral polymerases and also to be incorporated in the place of standard nucleotides in progeny viral RNA during replication (Ferrer-Ortega et al. 2010; Moreno et al. 2011; Perales et al.2011). Amongst mutagenic NAs, ribavirin is one of the best-known models which can cause the extinction of virus populations as reported in recent studies on HCV, *West Nile virus*, *Hantaan virus* and FMDV (Lazaro 2011, Moreno et al. 2011, Perales et al.2011).5-FU and 5-Azacytidine (5-AZA) is another important mutagenic NA, whose LM effects were reported to have extinguished populations of FMDV (Sierra et al. 2010), LCMV (Grande-Perez et al. 2002) and HIV (Dapp et al. 2009).

CONCLUSION

Outcomes of research have shown that many viruses play an important role in the emergence of severe infectious disease. As a result of investigations aimed at increasing knowledge about viruses and pathogenesis, a large of number antiviral drug have been developed and presented for using the treatment from past until present. Currently, antivirals are widely used all over the world for the treatment of viral disease. However, the growing resistance to antivirals across the globe presents an increasing threat to the efficacy of available treatment for various viral infections such as HCV, HIV and others. Novel therapies for RNA viruses are urgently needed to counter the threat from increased antiviral drug resistance. Lethal mutagenesis is one of the important alternative strategies studied by researchers. It seems likely that, by using this lethal mutagenesis approach, a whole range of new antiviral strategies can be generated, and a greater understanding of viral population dynamics can be facilitated.Although the molecular mechanism leading to lethal mutagenesis is not fully understood, it is recommended that further investigation should be undertaken to discover or create new mutagenic agents.New antiviral protocols should also be designed with a view to decreasing viral load, facilitating clearance by the immune system, or eliminating viruses.

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