

Antiviral Medications and Plasma Efficacy in Treating Ebola Patients: A Systematic Review

Paulchris Okpala¹, Frances Okpala² and Sarah Patterson³

Abstract

In 1976, the first outbreak of Ebola hemorrhagic fever (Ebola) occurred in Africa. Ebola is an acute viral syndrome that is characterized by fever and bleeding diathesis. Another distinct characteristic of Ebola is its high mortality in human and nonhuman primates, which, in turn, is caused by the Ebola virus which belongs to the viral family Filoviridae. Ebola is highly virulent, considered as a fifth-category notifiable communicable disease just like the Marburg virus. There is a lack of knowledge about the exact origin, location, and natural reservoir of Ebola virus, but it is believed that it was first transmitted to humans by animals, particularly, fruit bats. There is no fully accepted treatment for Ebola at this point in time. However, it is notable that since Ebola was first discovered in Sudan and Congo, researchers had been seeking to find a cure. Among the treatments currently being studied are antiviral and vaccines. Using the literature review method, this paper assesses current research on antiviral medications for Ebola and their plasma efficacy. ZMapp is considered to present the highest efficacy.

Keywords: Gene Therapy, Hemophilia, Gene Disease, Ebola

1 Introduction

Ebola hemorrhagic fever (Ebola) is an acute viral syndrome that is manifested through fever and ensuing bleeding diathesis [1]. Ebola, defined by its high mortality in human and nonhuman primates, is caused by the Ebola virus which is a “lipid-enveloped, negatively stranded RNA virus that belongs to the viral family Filoviridae” [2]. Ebola fever was first reported in 1976, during two simultaneous cases in southern Sudan and the Democratic Republic of Congo (formerly Zaire). The Ebola virus classified as a fifth-

¹DHSc, MHA, MPA, RCP, CRT, .T Still University of Health Sciences, Doctor of Health Sciences Alumni

²B.S., MSHR (in progress), Bellevue University of Health Sciences, B.S., Behavioral Science

³B.S (in progress), ³California State University San Bernardino, B.S., Health Care Administration

category notifiable communicable disease as is with other diseases such as the Marburg virus. The genus Ebola viruses are divided into five subtypes: Zaire (EBOV), Sudan (SUDV), Bundibugyo (BDBV), Tai Forest (TAFV) and Reston (RESTV) (Feldmann & Geisbert, 2012) Except for RESTV, all of these viruses cause disease in humans with each subtype having its own different biologic characteristic and virulence.

At this point, there is no clear understanding yet about the exact origin, location, and natural reservoir of Ebola virus, albeit studies have shown that the virus is zoonotic and fruit bats could be responsible for its spread. Treatments are currently at the experimental stage. For instance, post-exposure, non-antiviral strategies are classified into various categories, depending on their target of action [3]. These include (i) recombinant nematode anticoagulant protein c2 (rNAPc2) [12] and recombinant human activated protein C (rhAPC) that seek to treat clinical symptoms of coagulopathy and sepsis that infect Ebola patients although not exclusively; (ii) small, interfering RNAs (siRNAs) including positively charged phosphorodiamidate morpholino oligomers (PMO plus); and (iii) monoclonal antibodies (mAbs) used for suppressing viremia and virus spread [3]. There are also several vaccine candidates. The most viable ones are the (i) “rVSV (recombinant vesicular stomatitis virus) + EBOV-Z-GP (glycoprotein), rRABV (recombinant rabies virus) + EBOV-Z-GP, rAd5 (recombinant adenovirus serotype 5) + EBOV-Z-GP, VLP (virus-like particles) + EBOV-Z-GP, rHPiV3 (recombinant human parainfluenza virus type 3) + EBOV-Z-GP, rCMV (recombinant cytomegalovirus) + EBOV-Z-NP (nucleoprotein)” and (ii) rEBOV (recombinant Ebola virus) subunit vaccine + TLR (toll-like receptor) agonist [5]

Fairly recently, antivirals have been studied for treatment of Ebola. Albeit interferon was discovered in the late 1950s, it has not been widely used medically largely because of its severe side effects [6]. However, interferon has been combined with ribavirin for the treatment of viral infections such as hepatitis C. This paper presents a systematic review of the efficacy of antiviral treatments in line for Ebola treatment and their plasma efficacy. This is an important contribution to existing literature considering the fact that Ebola is currently affecting several countries in Africa and it has also affected some individuals in other countries. As such, the threat of the virus spreading to other countries and causing a global catastrophe is very real. Thus, finding the most effective treatment to cure patients will not only increase survival rate but also prevent the virus from causing global fear.

1.1 Study Design

This study uses the structured or systematic literature review method. An effective literature review is a “systematic and explicit methodology to identify, select and critically evaluate relevant studies, and collect and analyze the data emerging from the studies included in it” [7] Therefore, the primary goal of this literature review is to shed light about treatments being developed for Ebola fever specifically in the form of antiviral medications. Notably, a systematic literature review enables better understanding about the accomplishments achieved in studies on Ebola virus treatments. Therefore, a benefit derived from conducting a systematic literature review is the identification and analysis of extant literature on Ebola fever antiviral medications as well as their efficacy.

Systematic literature reviews provide evidence-based insights in order to facilitate the development of policies and guidelines in relation to the phenomenon being investigated or to expand on extant literature, among many other things. The end goal of this systematic review is to synthesize findings of existing studies for the purpose of

addressing the research questions and objectives. In light of these, this systematic literature review encompasses only clinical trials and experiments. Only articles published within the past decade were considered for this literature review to ensure that only relevant and new researches are covered.

2 Literature Search Method

The databases searched were CINAHL Plus, MEDLINE, EBSCOhost, ScienceDirect and Cochrane Database of Systematic Reviews. Search terms used were “Ebola virus,” “Ebola fever,” “antiviral” and “efficacy.” Figure 1 below shows the flow chart summarizing the search terms used and the results generated. Other peer-reviewed journals were retrieved for the purpose of cross-referencing so that this literature review could provide deeper discussion about Ebola fever antiviral treatments. These cross-referenced articles were used only to provide further information and support to the five studies that were ultimately selected for this systematic review.

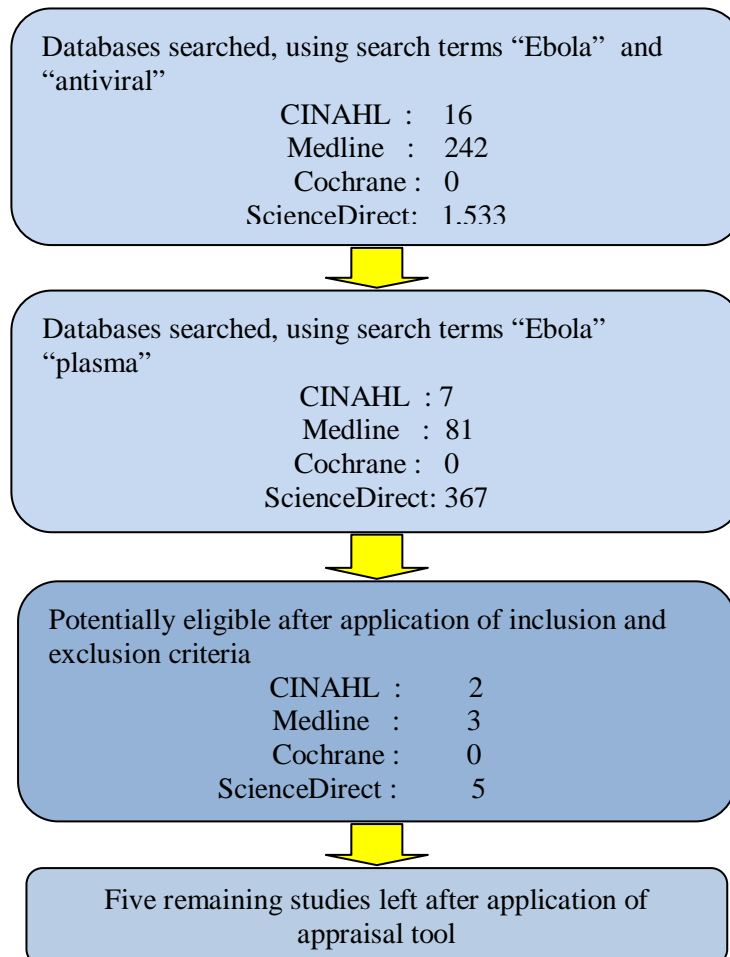


Figure 1: Flowchart of Literature Search

2.1 Inclusion and Exclusion Criteria

The systematic literature itself is supported by many studies because of the need to discuss the current state of research on Ebola. However, only five studies are reviewed in details. This is due to cross-referencing and searching from other peer-reviewed publications so that an overall discussion about Ebola treatments may be provided. This systematic literature review strictly used inclusion and exclusion criteria to ascertain that only quality studies are investigated. The following are the said criteria.

2.1.1 Inclusion criteria

Studies were included in the proposed literature review if they (i) were published in the English language; (ii) full-text articles; (iii) were empirical studies and systematic reviews; (iv) focused on antiviral medications and plasma efficacy; (v) were published between 2004 and 2014; and (vi) were published in peer-reviewed journals.

2.1.2 Exclusion criteria

Studies were excluded if they (i) were not full-text articles; (ii) were not empirical studies; (iii) were published before 2004; (iv) were conducted in unethical manners.

2.2 Analysis Tool

There is no standard set of criteria used for the analysis and evaluation of quality in experimental studies. Hence, the criteria used to analyze the methodological quality of the five studies were based on 12 criteria of assessment established by Harden, Garcia, Oliver, Rees, Shepherd [8] . In all, there are 13 criteria used for the analyses of the five studies. These criteria pertain to clarity of reporting, robustness of study methods, and sufficiency of response rate. Table 1 below summarizes the critical appraisal of the five articles. Each criterion met by the study was scored a single point, "1." Thus, the total quality score for the five studies should range from zero to 13.

Table 2: Critical Appraisal Summary

Study	Clear Reporting	Robustness	Total Quality
1 Qiu, X., Wong, G., Audet, J., Bello, A., Fernando, L., Alimonti, J. B., & ... Kobinger, G. P. (2014). Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. <i>Nature (London)</i> , 514(7520), 47-53.	7	5	12
2. Oestereich, L., Lüdtker, A., Wurra, S., Rieger, T., Muñoz-Fontelaa, C & Günther, S. (2014). Successful treatment of advanced Ebola virus infection with T-705 (favipiravir) in a small animal model. <i>Antiviral Research</i> 105, 17–21.	7	4	11
3. Aman, M.J. et al. (2009). Development of a broad-spectrum antiviral with activity against Ebola virus. <i>Antiviral Research</i> 83, (3), 245–251.	6	5	11
4. Iversen, P. L., Warren, T. K., Wells, J. B., Garza, N. L., Mourich, D. V., Welch, L. S., & ... Bavari, S. (2012). Discovery and early development of AVI-7537 and AVI-7288 for the treatment of Ebola virus and Marburg virus infections. <i>Viruses</i> , 4(11), 2806-2830. doi:10.3390/v4112806	7	5	12
5. Dye, J. M., Herbert, A. S., Kuehne, A. I., Barth, J. F., Muhammad, M. A., Zak, S. E., & ... Pratt, W. D. (2012). Postexposure antibody prophylaxis protects nonhuman primates from filovirus disease. <i>Proceedings Of The National Academy Of Sciences Of The United States Of America</i> , 109(13), 5034-5039.	6	5	11

2.2.1 Synthesis of Findings

The neutralization of antibody-based therapies have been widely-used for the treatment of post-exposure infection considering that they directly target the virion and cut off virus replication at the very early stage of viral entry. Since the first outbreak of Ebola in 1976, antivirals have been investigated for treatment options, from convalescent serum to

monoclonal antibody and from single antibody treatment to antibody cocktail.

VI-7537 and AVI-7288. In 2012, there were no approved treatments for filovirus infections. [9] reported their development of antisense Phosphorodiamidate Morpholino Oligomers (PMOs) AVI-6002, comprised of AVI-7357 and AVI-7539; and, AVI-6003, composed of AVI-7287 and AVI-7288, that targeted Ebola virus and Marburg virus respectively. Their development entailed the identification of optimal transcript binding sites for PMO-based RNA-therapeutics which they followed through the screening for effective viral gene target in mouse and guinea pig models used in adaption of viral isolates. The researchers then tested an evolution of chemical modifications, starting with simple Phosphorodiamidate Morpholino Oligomers (PMO) to transition to cell-penetrating peptide conjugated PMOs (PPMO) and ending with PMOplus that contained positively charged linkages in the PMO structure (Iverson, et al., 2012). The initial lead compounds combined two agents that targeted separate genes. The researchers then selected a single agent for treatment of each virus was selected, with the AVI-7537 targeting the VP24 gene of Ebola virus and AVI-7288 targeting NP of Marburg virus.

The researchers tested their discoveries on mouse and guinea pig lethal challenge models, an approach widely used in drug development for many therapeutics. Their study used both the mouse and guinea pig and assessed two related viruses. They found distinct differences in their studies, discovering that VP35 is a crucial target in Ebola virus but not in Marburg virus while the combination of VP24 and VP35 was effective for Ravn virus infected mouse model but not for the Marburg Musoke infected guinea pig. Nevertheless, the researchers were optimistic about the reproducible efficacy of the VP35 and VP24 combination for Ebola in the mouse, pig and nonhuman primate. During the screening of these models, the researchers operated on the hypothesis that the “evolution of these viruses would lead to elimination of less important genes and all targets are likely to be equally active” [9]. They came to the conclusion that inhibiting expression of some viral genes is more significant than others. Moreover, variations in “transcript target abundance, accessibility of the viral RNA target, the half-life of the resulting expressed viral protein, and subcellular localization of the viral product” provide explanations regarding certain genes are less effective when targeted by the PMO. Notably, the optimal viral gene target is crucial for viral growth as well as the role it plays in interacting with the host, especially the immune response.

Antibody Prophylaxis. Using antibodies for postexposure treatment of hemorrhagic fever virus infection has not been fully successful [10]. Nevertheless, the passive transfer of serum collected from survivors of Junin virus or Lassa virus had been shown to be effective as long as treatments are given soon after infection [10]. However, it is important to note that experiments on the passive transfer of serum has had a high failure rate for the treatment of filovirus infections, including, Ebola (Dye, et al., 2012). Consequently, antibody therapies seeking to prevent or limit filovirus infections have not gained widespread interest among researchers mainly because of the high failure rates. Dye, et al. (2012) were able to overcome the weaknesses of this potential intervention by leveraging the use of antibody from nonhuman primates (NHPs) that survived exposure to filoviruses under controlled conditions. Through the use of concentrated, polyclonal IgG antibody from these survivors, they were able to treat filovirus-infected NHPs through multiple doses administered over the clinical phase of disease. For example, the Marburg virus (MARV)-infected NHPs were administered 15 to 30 minutes postexposure with virus-specific IgG, with follow on treatments on the fourth and eighth days after exposure [10]. The postexposure IgG treatment was thoroughly protective such that the NHPs did

not show signs of disease or detectable viremia. The MARV-specific IgM antibody responses were created and all NHPs survived rechallenge with MARV. This indicates that they were all able to produce an immune response to virus replication. The researchers conducted subsequent studies, through which NHPs were infected with MARV or Ebola virus (EBOV). Treatments were delayed for 48 hours, with additional treatments on the fourth and eighth days after exposure. The delayed treatments protected both MARV- and EBOV-challenged NHPs. In these studies, two of the three IgG-treated NHPs did not manifest any clinical signs of illness, but the third NHP developed mild and delayed signs of disease yet fully recovered afterwards. These studies clearly show that postexposure antibody treatments are effective in protecting NHPs and represent options for filovirus therapies for human use.

FGI-106. [11] reported their discovery of a new, broad-spectrum small molecule inhibitor called the FGI-106 which has inhibitory activities against multiple and unrelated VHF, including Ebola virus, Rift Valley Fever (a Bunyavirus) and Dengue virus (a Flavivirus). After conducting exploratory testing, the researchers discovered that the FGI-106 also has inhibitory activities against other viral pathogens such as HIV (a retrovirus) and hepatitis C virus (a Flavivirus). [11] The FGI-106 was later discovered to consistently inhibit Ebola virus replication in a dose-dependent manner. The researchers confirmed its antiviral activity through the use of the Ebola virus plaque enumeration as a direct measure of viral replication.

According to [11] treatment with 2 μ M FGI-106 mediated a four-log reduction in infectious viral titers related to matched controls, “with an EC₉₀ for inhibition of viral killing of host cells estimated to be 0.6 μ M.Z.” Whereas the broad-spectrum antiviral activity in cell-based assays was unexpected, animal-based efficacy is actually a more considerable challenge. Before initiating investigation of FGI-106 in mice, the researchers first determined the bioavailability of the molecule, especially within relevant target organs. Because the researchers wanted to focus on Ebola virus, they conducted a series of pharmacokinetic studies to assess serum levels of FGI-106 and accumulation within organs that are usually targeted by Ebola virus, namely, the kidney, liver and spleen [11]. Subsequently, the injected FGI-106 at 3 mg/kg to C57BL/6 mice and serum samples were collected at different time intervals at 0, 5, 15, 30, 60, 180 and 300 minutes. The purpose here was to analyze FGI-106 levels using tandem mass spectrometry. Through these studies, the researchers found that there was maximal concentration five minutes after injection, with a serum half-life of roughly 1.8 hours [11]. This means to say that there is rapid depletion of the serum, thereby posing the question of whether FGI-106 might efficiently distribute into the spleen, liver, and kidney.

To address this concern, groups of three C57BL/6 mice were administered a 3 mg/kg intravenous dose of FGI-106 for six hours [11]. This time frame was selected based on previous studies showing that six hours is adequate to allow a drug to transit from the blood and accumulate within organs [11]. The researchers sacrificed the animals in order to harvest their lung, liver, kidney and spleen for mass spectrometry-based assessment of FGI-106 concentrations. The studies show that the FGI-106 entered the said organs at levels ranging from 19.5 (spleen) to 43.1 μ g/g (kidney). These results show that there is potential promise for efficacy in animals in light of cell-based assays, which had estimated an EC₉₀ of 0.004 μ g/g for Ebola virus.

T-705 (Favipiravir). Oestereich, Lütke, Wurra, Rieger, Muñoz-Fontelaa & Günther (2014), examined the efficacy of the pyrazinecarboxamide derivative T-705 (favipiravir) against Zaire Ebola virus (EBOV) *in vitro* and *in vivo*. T-705 (favipiravir) was first

discovered in 2002 by Toyama Chemicals (Japan) as an inhibitor of influenza virus replication and is presently at the late stage clinical development for the treatment of flu [12]. The T-705 is converted by host enzymes to T-705-ribofuranosyl-5'-triphosphate and “acts as a nucleotide analog that selectively inhibits the viral RNA-dependent RNA polymerase or causes lethal mutagenesis upon incorporation into the virus RNA” [12].

Aside from influenza virus, T-705 has been found to have potent antiviral activity against other segmented negative-strand RNA viruses such as arena- and bunyaviruses in vitro and in vivo (Oestereich, et al., 2014). It has also been found to be active against positive-strand RNA viruses such as noro- and flaviviruses [12]. According to the study conducted by Oestereich, et al. (2014), the T-705 suppresses replication of Zaire EBOV in cell culture by 4 log units with an IC₉₀ of 110 µM. Mice that do not have the type I interferon receptor (IFNAR^{-/-}) were used as in vivo model for Zaire EBOV-induced disease. Here, the start of T-705 administration at day six after infection “induced rapid virus clearance, reduced biochemical parameters of disease severity, and prevented a lethal outcome in 100% of the animals” [12]. The researchers suggest that T-705 be considered for the treatment of Ebola fever.

ZMapp. One of the most recent studies on antiviral medications for Ebola has been the ZMapp (Qiu, Wong, Audet, Bello, Fernando, Alimonti & Kobinger, 2014). This study was an experiment on various combinations of antibodies from MB-003 and ZMAb in NHPs, and the selected formulation with the best preventive effect, termed ZMapp, was comprised of c13C6 from MB-003 and two antibodies, 2G4 and 4G7, from ZMAb [13]. All three mAbs “recognized conformational epitopes located on GP2 or the stem region of GP trimer, while the remaining three antibodies from MB-003 and ZMAb were bound to the trimer head” [13]

The researchers administered three doses of ZMapp to challenged NHPs on the fifth, eighth and 11th days after exposure at 50 mg/kg per dose [13]. All of the NHPs survived. Yet, the animals showed Ebola symptoms and detectable viremia at five days before treatment with ZMapp; however, viral load in the blood 21 days post-infection [13]. The importance of this study is that it demonstrates how people with Ebola may be saved through antiviral medications even after symptoms have appeared. ZMapp also shows inhibitory activity against the epidemic strain in cell culture and some actual cases of people recovering from ZMapp have been documented [13]. Therefore, it is considered as the most promising antiviral drug against Ebola virus.

3 Conclusion

Since the first outbreak in 1976, there have been no known treatments for Ebola fever. However, since that time, researchers have tirelessly sought to find such treatments. Consequently, different types of therapies have been investigated, including, the use of vaccines and antivirals. Using the systematic literature review method, this paper makes an assessment of current research on antivirals against Ebola fever. Based on this review, it has been determined that ZMapp has the highest potential in terms of efficacy.

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