Chapter 11

Treatment of Ebola Virus Disease: Therapeutic Agents

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“We are in an unusual situation in this outbreak. We have a disease with a high fatality rate without any proven treatment or vaccine,” says Dr Marie-Paule Kieny, assistant director-general at the World Health Organization (WHO). “We need to ask the medical ethicists to give us guidance on what the responsible thing to do is.”

WHO published a report on the ethics of using unregistered interventions to treat Ebola virus disease, where they concluded “In the particular context of the current Ebola outbreak in West Africa, it is ethically acceptable to offer unproven interventions that have shown promising results in the laboratory and in the animal models but have not yet been evaluated for safety and efficacy in humans as potential treatment or prevention.”

THE EVOLUTION OF ANTIVIRAL AGENTS

Viruses, unlike other microbes, are not made of cells. They are actually genetic material wrapped in a case of proteins. They have no function as long as they are outside of cells. Viruses enter cells when their protein case comes into contact with the outer surface of a human cell, and only then, they can take over cell function. The virus, once inside a cell, hijacks the machinery of a cell to replicate, and eventually replicates so many copies that the cells burst and release many copies of that virus only to wreak havoc in adjacent cells.

William Herman Prusoff (June 25, 1920–April 3, 2011), a pharmacologist, was among the first to develop an effective antiviral agent, approved in the
1960s by Food and Drug Administration (FDA). Dr Prusoff spent most of his career in studying thymidine, a building block of deoxyribonucleic acid (DNA). In the latter part of the 1950s, for the first time a clinical antiviral drug was shown as having selective antiviral activity when used properly. William Prusoff developed the first thymidine look-alike, 5-iododeoxyuridine, and Professor Herbert E. Kaufman showed the compound to be an effective treatment for herpes of the eye by disrupting the virus’ ability to reproduce in topical form. It worked by acting as a decoy genetic material. This decoy material does not allow the cells machinery to work properly, hence rendering the virus ineffective and serving as a treatment. So if a virus was replicating in a human, the drug would stop it in its tracks. This discovery was a big scientific breakthrough as it opened doors for a new era of antiviral therapy.

A drug by the name of acyclovir was the next big antiviral agent in the game. The drug worked by stopping the virus from using its mechanisms to replicate its genome. It was derived from a sponge that resides in the Caribbean seas, Cryptotethya crypta. Howard Schaffer and Robert Vince codiscovered the compound, which proved to have promising antiviral activity, and patented by Schaffer in 1979. A researcher by the name of Richard Whitely would be the first to use the drug in patients. Acyclovir is still used today, especially for infection by Herpesviridae.

Therapeutic development was a challenge, especially for specific agents targeting Flavivirus group, Ebola, and Marburg viruses even as early as in the 1990s. On the other hand, other viral hemorrhagic fevers like Lassa fever and Crimean–Congo hemorrhagic fever responded to a drug called ribavirin. Ribavirin works by acting as a decoy viral genome and, when incorporated into the genetic material, does not allow for further manufacturing of viral copies. Since ribavirin was effective against other hemorrhagic fevers, it made sense to run an experiment to see if it had potential use as a therapeutic against Ebola virus. Unfortunately, looking at its activity in laboratory viral cultures, it proved to be useless against the Ebola virus.

In August of 2011, the Massachusetts Institute of Technology (MIT) discovered a drug that would only attach virally infected cells. They would call this drug Double-stranded RNA Activated Caspase Oligomerizer (DRACO). The drug would program only virally infected cells to die. It proved to work against all 15 viruses it was tested against, including viruses like polio virus and dengue fever.

**ANTIVIRAL AGENTS FOR EBOLA VIRUS**

Now that the Ebola virus disease has spread across West Africa and to different continents, therapeutic intervention is desperately needed. Since Ebola virus disease is extremely rare, its rarity has not allowed for proper testing of effective treatment. Now that were in the largest outbreak to date and the entire globe is at risk, rather than just a remote village in Africa, the race to develop drugs against Ebola virus is on.
The most notable antiviral agent to date is the drug brincidofovir discovered by Dr Hostetler, a professor of medicine at the University of California, San Diego School of Medicine. Chimerix, founded by Hostetler in 2002, announced that brincidofovir would be one of two investigational agents clinically tested in West Africa in patients with Ebola virus disease. This drug had actually been developed for the treatment of another virus, cytomegalovirus, which is well known for its infection in patients with weak immune systems. It is currently in Phase 3 trial for the treatment of adenovirus and cytomegalovirus after proving to have a good safety profile and works by stopping the replication process of viruses, specifically DNA viruses. Paradoxically, it has been shown to be effective against Ebola virus, which is an RNA virus. Essentially, the reason it interferes with Ebola virus survival is unknown. Brincidofovir has also been stockpiled by the United States as potential use as a biodefense agent, especially against smallpox.

How exactly did this drug come into the Ebola virus disease scene? The Center for Disease Control and Prevention (CDC) and the National Institutes of Health asked a drug manufacturer, Chimerix, to give them large quantities of antiviral drugs to test in virus cultures in a laboratory setting. Incidentally, brincidofovir was a potent inhibitor of Ebola virus in culture. In October 2014, the FDA provided emergency authorization for use of this agent in patients with Ebola virus disease. Although there are many other antiviral agents being developed for the Ebola virus disease, brincidofovir had moved ahead of the pack because of its established reputation. The drug has already been used in hundreds of human subjects including children, and health professionals know its properties. Brincidofovir has been used in two patients thus far, Thomas Duncan and Ashoka Mukpo and, although no evidence reports its efficacy in humans, the drug company is ready to start trials for patients. Unfortunately, Duncan received the therapeutic agent when he was already critically ill and passed away. Ashoka Mukpo fortunately survived.

Another drug developed by Tekmira (Tekmira Pharmaceuticals Corporation) called TKM-Ebola, is already being tested in a Phase 1 trial. However, the trial was put on a partial hold because some subjects had a flu-like response to the medication. The medication can still be used with expanded access in patients with Ebola virus disease.

Other investigators have proposed that since Ebola virus disease causes the victims to bleed, why not just stop the bleeding? With that in mind, a publication in 2012 described using a drug that was derived from nematodes. This drug works by acting on the mechanisms in blood that control the balance of its inherent clotting properties. Since Ebola virus disease patients are likely to bleed, it made sense to use a drug that gives the blood a tendency to clot in order to counterbalance the bleeding. The drug resulted in survival in three of nine rhesus monkeys. In the same publication, the development of two new therapeutic agents AVI-7537 and AVI-7288 were described. Both of these agents are actually small strands of genetic material developed by Sarepta Therapeutics Inc. These drugs worked by stopping the virus from making important products.
essential to the virus’s inherent function, therefore, rendering it nonviable. The drug had a promising safety profile in one study, but further studies could not be done, possibly due to a lack of funding.\textsuperscript{10}

\section*{VACCINES}

Vaccines, in general, are used to prevent infection. Vaccines are theoretically effective before an individual is infected with an agent, in this case, Ebola virus. Now how are vaccines made? And how do they work? The answer lies in the structure of the Ebola virus.

The Ebola virus, as mentioned above, is made of genetic material wrapped in a protein coat. The components, when broken down, are essentially harmless to humans, it is the entirety of the intact virus inside that raises hell during an infection. The body recognizes the various components of the virus and produces a reaction to those components. With those facts in mind, why not have some drug companies obtain samples of Ebola virus, break the virus down into harmless components, and inject the “component” particles into humans, so that we can mount a reaction to them?

These are the basic questions asked when a vaccine needs to be made. Once the body is exposed to Ebola virus particles, it will make antibodies that react to them, as if it is actually fighting the virus. Antibodies need to be “specific” when attacking an invader. The body has to manufacture a different set of them for each unique infection. Once the body has fought off the infection, it “learns or remembers” the structure of the invader it has fought off, so that, in the event it invades again, there is stored ammunition against it. A vaccine is a mock of the virus that does not cause infection, but do allow the body to manufacture the necessary ammunition in the event of a true Ebola infection ensues.

The U.S. National Institute of Allergy and Infectious Diseases (NIAID) and GlaxoSmithKline (GSK) have teamed up to create vaccines that are currently being tested.\textsuperscript{11} The vaccines have completed their first phase of testing on 20 healthy individuals in the United States to determine their safety and immune response. This vaccine was actually made from a virus that causes colds in monkeys. The cold-causing adenovirus was engineered to express components of Ebola virus, hence “mimicking” Ebola virus. A similar vaccine was also made for another strain of Ebola virus and is currently being tested in Mali, the United Kingdom, and Switzerland. The investigators also tested different doses in the individuals and found that there are more antibodies made when a higher dose is given compared to a lower one.
perhaps the most challenging are the properties of the intruder itself. A good example of this is malaria. The malaria parasite, which is transmitted by mosquitoes to humans, infects over a couple million people and kills just under a million people per year. Scientists have been searching for a vaccine for years, but the solution has evaded them. The real issue lies in a mechanism the parasite possesses called antigenic variation. Antigenic variation means that the parasite is able to change the way it looks to the immune system. Anytime the immune system attempts to mount a response to the parasite, the parasite will change itself like a chameleon (*Chamaeleonidae*). The lizard has the ability to change colors according to the surrounding habitat and go undetected. Similarly, the malaria parasite can go totally undetected, therefore, continuing to thrive and devour its host. Since vaccines are basically decoy intruder molecules, developing a vaccine for something that has no true constant recognizable identity to the immune system remains a challenge. Because of their complex life cycle, complicated genome, and the poorly understood response of the human immune system to malaria, the fight to develop a vaccine continues.

Another virus that we just cannot seem to develop a vaccine for is human immunodeficiency virus (HIV), despite it being around for over 30 years. Even with the NIAID’s worldwide effort to create a vaccine, there have been no effective vaccine. HIV is a bit different from most viruses, in the sense that the human body can never rid itself of HIV. Other viruses, like the flu and Ebola viruses, can be cleared by the human system, and often patients develop immunity to the viruses. The antibodies that the body produces against HIV are not effective in fighting the virus and the other arms of the immune system that do not use antibodies to fight infection are attacked by the HIV virus itself. Not only is the immune system not effective against the virus, but HIV attacks the immune system itself, making the body more prone to other infections, which is often the cause of death in HIV-positive patients. Even if a vaccine were to be developed, the complex life cycle of HIV poses a challenge because HIV is a “retrovirus.” Retroviridae have the unique ability of being able to incorporate their genome into the genome of the host. Meaning that HIV basically can hide within the genome of human cells, making it extremely difficult to target.

Now that the potential challenges have been explained, let us discuss the Ebola virus vaccine. The first and foremost issue is the rarity and combined lethality of the virus. The outbreaks tend to be small and in remote areas of Africa, usually self-limiting after a hundred cases or so. Subsequently, information, the priority of research and therapeutic development has not entered the priority of big player pharmaceuticals, at least until this outbreak. Another deterrent is the daunting process of development. Once someone has come up with a potential candidate vaccine, the rigorous process of testing and approval ensues. Some drug trials can take up to 10 years, if not longer. The safety and efficacy has to be thoroughly tested for the
vaccine to be approved. Although the process is long and arduous, it is in the best interest of vaccine recipients.

But what happens when the situation demands a vaccine for a deadly hemorrhagic virus spreading with no regard for life and has essentially become a global threat? Even with this current scenario, “fast-track” development projections for current vaccines are going to take a year or so. And although this is exceptionally fast in comparison to other therapeutic approvals, is it fast enough to stop or slow the Ebola virus disease? Only time will tell.

Although the current vaccine has proved to induce development of antibodies in humans, we still do not know that these antibodies are going to be effective. Is Ebola virus going to be like a chameleon and change itself to evade the immune response? We already know that the Ebola virus can target the immune system, rendering it ineffective. There are multiple strains of Ebola virus. Can a single vaccine protect against all strains? Can a vaccine be developed such as the quadrivalent flu vaccine which protects against two influenza A viruses and two influenza B viruses. What if the developed vaccines prove to not have a true efficacy after being deployed on a large scale? We may have to start from ground zero yet again.

ANTIBODIES FROM EBOLA VIRUS DISEASE SURVIVORS

Antibodies made by survivors of Ebola virus disease continue to circulate within the blood for an undetermined amount of time. These antibodies can be extracted from blood and given to patients who actively suffer from Ebola virus disease. The extracted product is called convalescent serum. In order to prepare this serum, blood must first be extracted from Ebola virus disease survivors. Once blood is collected, it must be spun in a centrifuge to separate the components. The heavier elements, including red and white blood cells and platelets, will naturally separate from the lighter component, plasma. It is the plasma that contains the convalescent serum. The plasma is then transfused into a suffering patient. In Kikwit, Democratic Republic of the Congo, between June 6 and 22, 1995, eight patients were transfused with blood donated by five convalescent patients who met the case definition used in Kikwit for Ebola virus disease. The donated blood was expected to contain antibodies against Ebola virus. All the transfusion recipients were tested positive for Ebola virus antigens just before the transfusion. The transfused patients had clinical symptoms similar to other Ebola virus disease patients during the epidemic. All were severely weak, four had hemorrhagic manifestations and two became comatose as their disease progressed. Compared to overall very high case fatality rate (80%) for Ebola virus disease epidemic in Kikwit and the fatality rates observed in other Ebola virus disease epidemics, only one of the patient who received the survivor antibodies died. The cause for this low fatality rate remained unexplained, and results are confounded by better care which was given to transfused patients as compared to those
treated in the initial phase of the epidemic, which could have contributed to the higher survival rate.15

ANTIBODIES MADE FROM OTHER ORGANISMS

Instead of waiting for a survivor to appear with antibodies, why not just infect other living organisms with Ebola and extract their antibodies? The first drug used experimentally against Ebola virus disease was an agent called ZMapp developed in the tobacco plant *Nicotiana benthamiana* in the bioproduction process known as “pharming.”16 ZMapp is a drug cocktail of antibodies made against Ebola Virus. Leaf Biopharmaceutical (LeafBio, Inc.), a San Diego-based arm of Mapp Biopharmaceutical is developing the composite drug.

An experiment published in *Nature* indicated that, if ZMapp treatment is initiated up to 5 days post-Ebola virus infection, the antibody cocktail is able to save 100% of rhesus macaques. In many animals, high fever, viremia, and abnormalities in blood count and blood chemistry were evident before ZMapp intervention. Elevated liver enzymes, mucosal hemorrhages, and generalized small bleeds, indicating the advanced disease could be reversed, leading to full recovery. ZMapp is cross-reactive with the Guinean variant of Ebola virus as demonstrated by enzyme-linked immunosorbent assay and neutralizing antibody assays, which is a test used to detect microbes. Further development of ZMapp for clinical use is based on its surpassing value over any other therapeutics described so far.17

Despite ZMapp promising to be a useful therapeutic agent as demonstrated in the experiment with rhesus monkeys, an enormous supply and demand issue has emerged. The reason for this shortage lies in the development process of ZMapp. To produce this drug, genes encoding the antibodies are inserted into viral vehicles, which are then used to deliver the genome to tobacco plants, and, in turn, this produces the antibodies from the delivered genes. The antibodies are then extracted and purified for development. This entire cycle takes a few months to complete. The amount of ZMapp needed for this current outbreak is severely outpaced by the spread of Ebola virus disease. Even once there is enough ZMapp made, its true effectiveness in humans has yet to be determined, although it has proved valuable in rhesus monkeys. It would have to go through a similar vigorous testing process like other vaccines. We can only hope that popular approval and large-scale production will catch up to the wrath of the current Ebola virus disease epidemic.

At this current point in time, there is no proven vaccine or treatment for Ebola virus disease. The current treatment is purely a supportive one. The therapeutic agents mentioned above have been used in several patients in the United States, and a majority of those patients have survived. More studies are required to know the toxicity profile for current experimental therapeutics.
REFERENCES


