Extracorporeal Virus Elimination for the Treatment of Severe Ebola Virus Disease – First Experience with Lectin Affinity Plasmapheresis

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Key Words

Ebola virus · Glycoprotein · Lectin affinity · Plasmapheresis

Abstract

Therapeutic options for Ebola virus disease (EVD) are currently limited to (1) best supportive care, and (2) evolving virus-specific therapies, resulting from decades of analyzing one of the world's deadliest diseases. Supportive care ranges from oral or intravenous rehydration therapy and anti-emetics in developing countries to much more extensive life-support interventions in resource-rich countries. Current EVD-specific therapies attempt to either interfere with the earliest steps of viral replication or to elicit a strong immune response against the virus. An entirely new approach is the extracorporeal elimination of viruses and viral glycoproteins by lectin affinity plasmapheresis. Herein, we report for the first time the successful and safe use of lectin affinity plasmapheresis in a patient with severe Ebola virus disease.

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Introduction

A year ago, a strain bearing 97% homology to the most pathogenic strain of Ebola virus (EBOV), Zaire, started to spread in Africa, primarily in the West African countries of Guinea, Sierra Leone, and Liberia. As of December 31, 2014, the World Health Organization (WHO) reported 20,206 cases, and 7,905 deaths [1]. Some authors estimate the current case fatality rate to be approximately 70% [2].

EBOV is an enveloped RNA virus, belonging to the Filoviridae family, which also includes the genera *Marpburgvirus*, and *Cuevavirus*. EBOV acts by vigorously crippling both innate and adaptive immunity, causing death in usually a matter of days [3]. EBOV also demonstrates multiple immune evasion features including: a comprehensive disabling of viral recognition by squelching interferon signaling, inactivation of antigen presentation by

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EBOV glycoproteins (GP) [4, 5], and vast lymphocyte apoptosis [6]. Immune evasion is enhanced by a process in which GP are shed into circulation directly from the EBOV-infected cells where they likely act as decoys in neutralizing antibodies [7]. In addition, shed GP have been shown to induce a massive release of cytokines and increased vascular permeability [8] which in turn leads to systemic inflammation, excessive volume loss, electrolyte disturbances, infectious, and bleeding complications, septic shock-like syndrome, multi-organ failure, and ultimately, death.

Notwithstanding scientific efforts and anecdotal reports of successful interventions, no EBOV-specific therapy has proved to be efficient to this point. Thus, best supportive care is the current treatment standard for Ebola virus disease (EVD) [9, 10]. By replacing the excessive volume and electrolyte deficits caused by severe diarrhea and vomiting, administering antiemetics, and by establishing an adequate anti-infective strategy to treat bacterial superinfection, mortality can be reduced in certain populations to about 40% in West Africa [11]. Including replacement therapy for failing organs appears to further lower the fatality rate. An entirely new approach is the extracorporeal elimination of viruses and viral GP by lectin affinity plasmapheresis (LAP).

Short Patient History

The patient, a 38-year-old male from Uganda, was admitted to our hospital on 10/3/2014. On 9/28/2014 he developed fever, and was tested positive for EBOV by semi-quantitative real-time PCR (qRT-PCR, initial CT value 31.61) [12]. On arrival, the patient was very weak, with fever, chills, gastrointestinal pain, emesis, and diarrhea. EBOV qRT-PCR yielded 1.84×10^8 copies/ml in plasma [12]. The viral load was determined according to a standard curve provided by the National EBOV Reference Laboratory in Marburg, Germany.

The intensive care therapy under Biosafety level 4 conditions has been described in detail elsewhere [12]. Based on reports that lectin affinity plasmapheresis (LAP) can reduce viral load in enveloped virus-infected patients [13] and in vitro data also showing successful reduction of EBOV in cell supernatants and primate blood (L. Barrientos, US Centers for Disease Control, personal communication), we discussed the possibility of using a LAP device (LAPD) in this life-threatening situation. Initial supportive therapy included balancing volume status, anti-infective, antiemetic, and anti-pyretic medication, as well as administering pain-relieving drugs [12]. The patient was treated with amiodarone from EVD day (d)1 (9/28/2014) until transferal as described previously [12] when it was stopped at the patient's request. In addition, Faviripavir was administered twice, but uptake of the substance was uncertain because of frequent vomiting [12]. On 10/8/2014 (EVD d9), continuous renal replacement therapy (CRRT) was

started subsequent to multi-organ failure (respiratory rate >40/min, anuric acute kidney injury, need for vasopressors, and high volume). In order to adjust for endothelial leakage, FX06 (peptide B β 15–42) was given from EVD d11 to d13 (10/8/2014 till 10/10/2014) [12]. After emergency approval by the regulatory authority (BfArM), and a positive vote by the local ethics committee on EVD d13, 5 days after initiation of dialysis, an LAP treatment was performed lasting 6.5 h. The objective of this therapeutic intervention was to lower the patient's circulating viral load and GP by their respective adsorption to the lectin-binding sites within the device.

A special approval according to §11 of the German Act on Medical Devices (Medizinproduktegesetz, MPG) was given by the Federal Institute for Drugs and Medical Devices (BfArM; Reference/Geschäfts-Zeichen: 5200–00660/14) for the use of the Hemopurifier®. The local ethics committee also agreed to the use of the device. The patient's informed consent was obtained after his recovery as the patient was intubated and sedated before the treatment day.

Methods

Lectin Affinity Plasmapheresis

Lectin affinity plasmapheresis therapy is based on the concept of affinity chromatography developed in the 1970s [14]. It combines plasma separation using a hollow fiber plasmafilter with virus capture via immobilized affinity agents residing in the extracapillary spaces of the plasma filter. Research has shown that a unique lectin protein (*Galanthus nivalis agglutinin*, GNA) from *Galanthus nivalis* (the common snowdrop) has a high affinity to the mannose-rich GP that are universal constituents on the surface of enveloped viruses [15, 16] to inter alia mediate entry into host cells. GP are also shed directly from infected cells [8].

As blood enters the plasmafilter, a portion of the plasma is forced through the pores of the membrane (≈ 200 nm) due to the blood side pressure. Because the hollow fiber bundle creates a resistance to the flow of blood, a pressure drop is created along the length of the device such that the blood-side pressure is higher at the blood inlet and lower at the blood outlet. This causes plasma to flow away from the blood and into the extracapillary space (where the affinity resin resides) along the proximal third of the fiber bundle. In the distal third of the fiber bundle, the pressure gradient is reversed; this causes the plasma to flow backward through the membrane recombining with the blood but without the viruses and GPs that have been bound by the GNA.

This technique does not result in the loss of any plasma since the plasma never leaves the device (the plasma ports are left capped). Also, in the submissions of summary human clinical data provided by the manufacturer of this device to the US and German medical device regulatory agencies, there has been no indication that any beneficial biomolecules are being adsorbed to any clinically significant level.

Renal Replacement Therapy

The dialysis procedure was performed using a multiFiltrate Ci-Ca® device (Fresenius Medical Care, Bad Homburg, Germany) equipped with the multiFiltrate Ci-Ca-cassette tubing system (Fresenius Medical Care, Bad Homburg, Germany) and the AV 1000S

Fig. 1. Method of lectin affinity plasmapheresis. **a** Binding of Ebola virions and shed/secreted Glycoproteins (GP) on the affinity matrix. GNA = Galanthus nivalis agglutinin. **b** Sketch of the plasmafilter showing separation of the blood in plasma and corpuscular components. Ebola virions (yellow) are passing through the

membrane towards the affinity matrix. Corpuscular blood components stay within the hollow fiber. **c** Sketch of the extracorporeal circuit showing the additional plasmafilter (LAPD) upstream of the hemofilter. LAPD = Lectin affinity plasmapheresis device (Hemopurifier®). **d** Picture of the running system.

dialyzer (Fresenius Medical Care, Bad Homburg, Germany). The LAP-device (Hemopurifier®, Aethlon Medical, San Diego, Calif., USA; for detailed specifications see supplement) was incorporated in the arterial line upstream of the dialyzer. The top of the LAP device was connected to the bottom of the dialyzer with an additional tubing system (fig. 1). Anticoagulation of the extracorporeal circuit was achieved using regional citrate anticoagulation. The multiFiltrate Ci-Ca instrument was set to maintain an ionized calcium level within the extracorporeal circuit of <0.3 mmol/l. To maintain systemic ionized calcium level within the patient of between 1.0 and 1.2 mmol/l, calcium chloride was infused automatically into the blood returning to the patients and systemic and extracorporeal ionized calcium levels were checked every 4 hours.

Post-dilution continuous venovenous hemodiafiltration was the mode of dialysis used. The prescribed dialysis modality was never modified to accommodate the LAP treatment. The treatment was well tolerated with no adverse events. There was no clotting, hemolysis or anaphylactic reaction noted during the single 6.5 h treatment period. After the treatment, the used LAP device was removed without complications during a short pause in the dialysis procedure. It was flushed with 1,000 ml 0.9% NaCl solution, and kept at 4°C until being transported to Marburg University's biosafety level 4 lab for virus quantification (see online supplement material; for all online suppl. material, see www.karger.com/doi/10.1159/000375229).

Elution and Viral Load Results

On EVD day 13, viral plasma load was 3.78×10^5 copies/ml 4 hours before LAP treatment was started. On EVD day 14 viral plasma load had dropped to 6.08×10^3 copies/ml as previously described [12].

Table 1 shows the results of a post-treatment elution protocol, which verified a calculated number of 253 million EBOV captured within the LAP-device during the treatment. Assuming a simplified one-compartment-model and an estimated plasma volume of 3 liters, the elimination of 8.43×10^4 copies/ml was achieved during the 6.5 h treatment. Two additional viral load measurements during the treatment phase showed a decrease from 2.29×10^5 copies/ml to 7.66×10^4 copies/ml.

Beginning with EVD day 13, a clinical improvement was noted and was accompanied by a further decrease in the plasma viral load and an increase of the EBOV-IgG-titer, which had already started to increase at d11 [12]. Six days after the LAP treatment, viral load was undetectable in the patient's plasma and the patient fully recovered.

Table 1. Results of the elution

Buffer	CT-value	Copies/ml	
PBS 1	27.89	9.63E+04	
PBS 2	27.67	1.12E+05	
PBS 3	27.74	1.07E+05	1.05E+05
aMM 1	28.15	7.96E+04	
aMM 2	28.44	6.51E+04	
aMM 3	28.54	6.03E+04	6.83E+04
AVL 1	24.72	8.90E+05	
AVL 2	24.3	1.19E+06	
AVL 3	24.52	1.03E+06	1.04E+06

Results of the plasmafilter-elution after the procedure, every single elution was measured in triplicates, elution volume 300 ml for PBS and 200 ml for aMM and AVL buffer. PBS = Phosphate Buffered Saline; aMM = alpha-methylmannoside; AVL = amp viral lysis buffer.

Discussion

The current mainstay of EVD treatment *in resource-rich countries* is best supportive care with aggressive fluid replacement, correction of electrolyte disturbances, and transfusion of blood products under intensive care settings. Despite reports of successful treatments [10, 17], even in this setting EVD always has the potential to progress to a severe critical illness with multi-organ failure and death. According to recent reports from Western Africa [18], approximately 60% of patients with EVD develop shock with oliguria or anuria. Despite the fact that acute kidney injury is associated with fatal outcomes in EVD [2], the need for renal replacement therapy is rarely reported during the recent outbreak. To date, dialysis has been initiated in only 3 patients [19]. EBOV is an elongated virus about 80 nm in diameter, and up to 1,000 nm in length with a molecular mass of 4,200 kD. It is highly unlikely that any significant quantities of this virus are removed during a dialysis session since the pore size of regular dialysis membranes are between 3 and 9 nm and have a molecular weight cutoff less than 40 kD [19]. A plasmafilter, as mentioned earlier, has a median pore size of approximately 200 nm through which EBOV are able to pass.

In general, experimental EVD therapy can be based upon three pillars: chemotherapy, passive/active immunotherapy, and, as proposed herein, extracorporeal virus elimination by affinity plasmapheresis. Regarding potential chemotherapeutic agents, one approach is an siRNA-cocktail (TKM-Ebola), which consists of siRNAs targeting viral polymerase, and proteins 24, and VP35 [20]. Filoviri-

dae-effective broad spectrum nucleoside analogues in animal models include BCX4430, brincidofovir, and favipiravir [21]. In vitro approaches include estrogen receptor (e.g., toremifene), and ion channel blockers (e.g., amilorone) [22]. A rather new supportive intervention addressing EBOV-induced coagulopathy and endothelial leakage is administration of the peptide B β 15–42 (FX06) [12, 23].

Within the realm of passive and active immunotherapy approaches, ZMapp, a cocktail of monoclonal antibodies, appears to give a survival advantage in non-human primates [24] but efficacy in humans has not yet been established. Currently, convalescent plasma or whole blood from survivors is considered an important intervention [25]. Also active immunotherapy trials with different vaccine platforms (e.g., recombinant vesicular stomatitis virus vector) are currently underway based on promising preclinical data [26].

All approaches share one proposition: they benefit from a reduction in viral load.

Rapidly lowering the number of circulating viruses, and GP frees up the available components (e.g., neutralizing antibodies) of the host immune response to attack the remaining viruses and eventually eradicate them wherever they reside. Also, every virus that is extracted from the circulation is no longer available to infect other cells. This is of great importance, since higher levels of viremia are associated with higher mortality [2, 27, 28]. Filtration and capture of circulating EBOV and GP represents an emerging device strategy for extracorporeal virus elimination. Furthermore, affinity binding to lectins provides a mechanism to address all strains of Ebola. The data contained in this case study represents a 'proof of concept' for extracorporeal virus removal in EVD. Furthermore, since the attachment epitope is the same, it is highly likely that GP are also extracted by this process.

We consider that LAP is a new option to expand best supportive care toward a virus-targeted therapy for EVD. One advantage this new concept might bring is the fact that only the virus is removed while other plasma components like antibodies remain in the patient. Thus, this treatment option will not interfere with an evolving immune response. Another advantage is, if a net viral clearance can be achieved by filtering, this would be without adding further drug toxicity or introducing new drug interaction risks. Also, it appears that, from the limited time we used LAP, no additional risk or harm was incurred during concurrent hemodialysis therapy. One factor that must be considered with the provision of this LAP technique is that, as time passes, more and more of the binding sites on the affinity resin will be occupied with virus or shed GP and the effi-

ciency of extraction will decline. At the moment there is insufficient experience to be able to determine the best time for treatment initiation or the optimal time to change out the cartridge for a new one. Further investigations are needed to address the best timing of initiation, the best treatment duration, and the possibility to enhance viral clearance with more devices used in series or in parallel.

The use of lectin affinity plasmapheresis is reported for the first time in the treatment of a critically ill patient with the severe Ebola virus disease. Although the impressive number of 253 million captured genomic copies of EBOV provides definitive evidence of the ability of this device to extract virus, the favorable outcome of the patient described in this case study cannot be attributed to the LAP treatment alone. It should be noted that, besides intensive supportive care for treatment of multi-organ failure and other experimental interventions, the device was used late in the course of the illness, and viral load was already declining before the start of the treatment [12]. As described by Chertow et al, the critical phase seems to be between days 7 and 12 with most deaths occurring in this time frame [18]. Also, nearly all patients who survived to day 13 ultimately lived [18]. For that reason, the real contribution of LAP is not clearly known.

However, this limited experience provides optimism that lectin affinity plasmapheresis is a promising new tool for the treatment of severe Ebola virus infection, and warrants further evaluation as well as technical development. The possibility to capture viruses efficiently out of an infected individual may also provide an interesting strategy for treating other viral diseases caused by enveloped viruses including (therapy resistant) HIV, hepatitis B or C (induced liver failure), and even influenza.

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Disclosure Statement

The authors declare that they have no competing interests.

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