Lectin Affinity Plasmapheresis for Glycoprotein and Virus Elimination

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INTRODUCTION AND OBJECTIVES

Enveloped viruses like Ebola, SARS, HIV and Influenza have been shown to shed and secret glycoproteins (GP) (Cook *et al.*). Immune evasion is enhanced by GP secreted and shed into circulation directly from Ebola infected cells. Soluble GP likely act as decoys in neutralizing antibodies (Ansari) and can induce a shift towards non-neutralizing antibody formation („antigenic subversion", Mohan *et al.*). In addition, shed GP have been shown to induce a massive release of cytokines by binding macrophages and dendritic cells and to increase vascular permeability (Escudero-Pérez *et al.*).

In October 2014 a patient with a Ebola Zaire strain (EBOV) infection was treated in our hospital. In order to reduce viral and GP load we performed a Lectin Affinity Plasmapheresis (LAP, first time worldwide in Ebola virus disease (EVD)). Viral and now GP removal by LAP were evaluated.

Methods

(1) LAP combines plasma separation with virus capture by Galanthus nivalis agglutinin (GNA) in the extracapillary spaces of the plasmafilter (see US Patent 20120037564 A1). GNA has a high affinity to GP that are universal constituents on the surface of enveloped viruses (Cosset *et al.*; Mahmood *et al.*). The LAP device (Hemopurifier®, Aethlon Medical, San Diego, USA) was incorporated in the arterial line upstream of the dialyzer. After approval by the German agency in charge (BfArM), and the local ethics committee LAP was performed safely on EVD day 13 (6,5 hours, Büttner *et al.*).

(2) Dialysis (post-dilution CVVHDF with regional citrate anticoagulation) was performed using a multiFiltrate Ci-Ca® device (Fresenius Medical Care (FMC), Bad Homburg, Germany) equipped with the multiFiltrate Ci-Ca-cassette tubing system (FMC) and the AV 1000S dialyzer (FMC).

(3) After treatment the device was flushed with 1000 ml NaCl 0,9%, stored in a refrigerator (4°C) for 10 days until transport to the National EBOV Reference Laboratory at Philipps-University in Marburg, Germany. There the LAP device was eluted according to the manufacturer's protocol. The eluted RNA was used for reverse transcription, and quantitative real-time PCR (qRT-PCR). In addition eluates were ultra-centrifuged, and virus pellets as well as supernatants were used for SDS gel electrophoresis followed by Western blotting with anti-GP antibody (Institute for Virology, Philipps University, Marburg, Germany).

Results

As shown by Western blotting soluble GP (sGP) and shed $GP_{1/2}$ were removed in addition to the elimination of 253.160.000 EBOV copies (qRT-PCRs). Viral load measurements during the treatment phase did show a 3-fold decrease. After day 13 the patient had improved steadily and finally fully recovered.

Conclusion

Especially because of the use late in EVD (d13), and already increasing EBOV-IgG titers as well as decreasing virus load the patient's favorable outcome cannot be attributed to LAP alone. However our data provide a proof of concept for Ebola GP and virus capture and warrant further examination of LAP in diseases caused by enveloped viruses.

References

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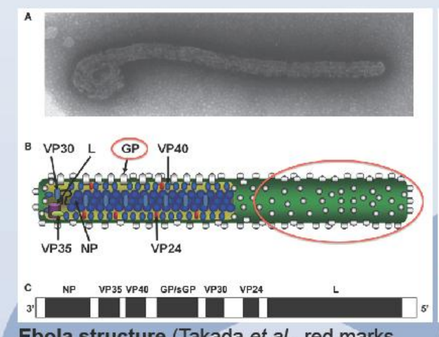
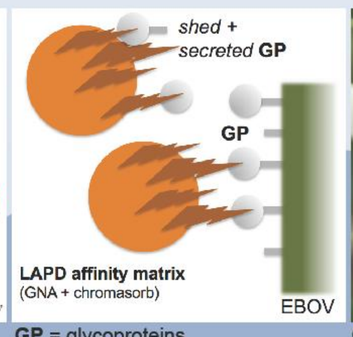
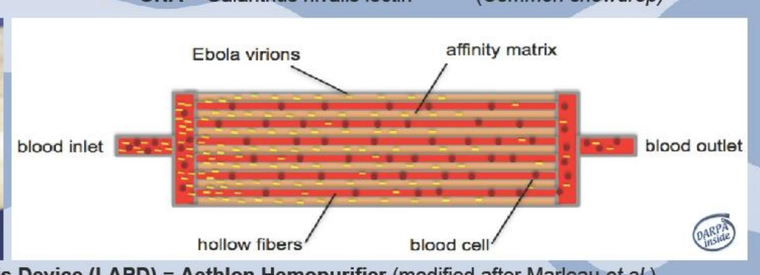


Ebola structure (Takada *et al.*, red marks added)

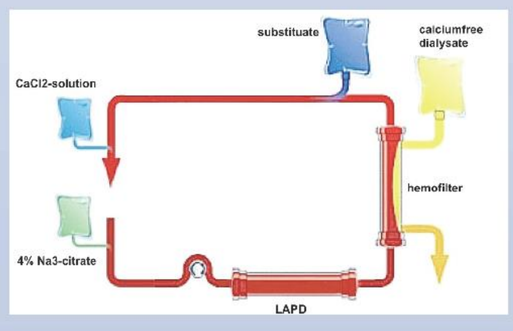


GP = glycoproteins
GNA = Galanthus nivalis lectin

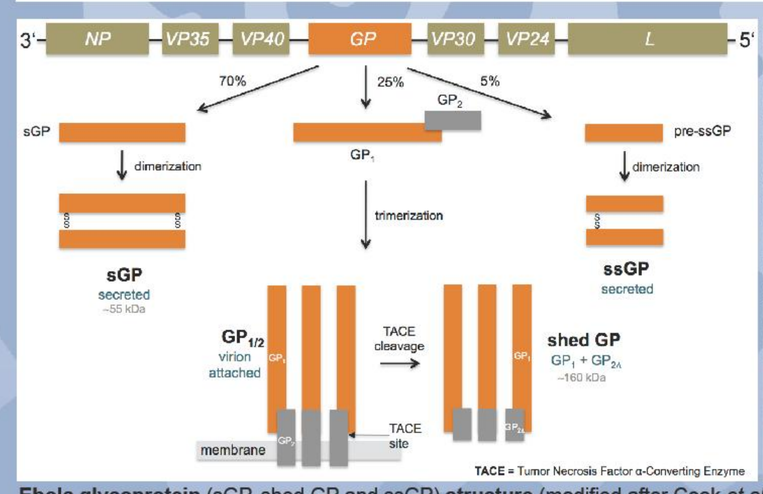
Galanthus nivalis
(*Common snowdrop*)



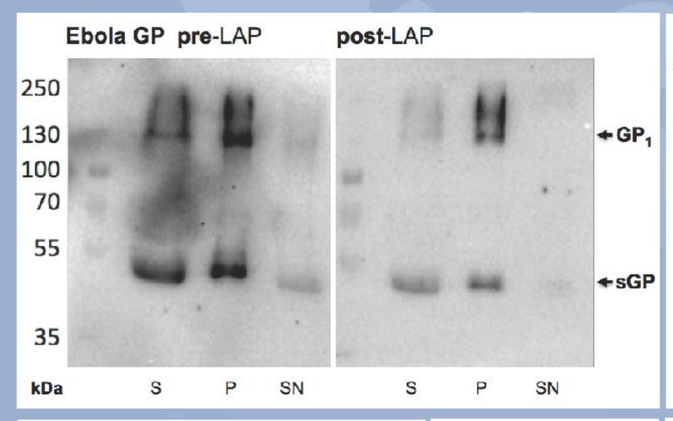
Lectin Affinity Plasmapheresis Device (LAPD) = Aethlon Hemopurifier (modified after Marleau *et al.*)

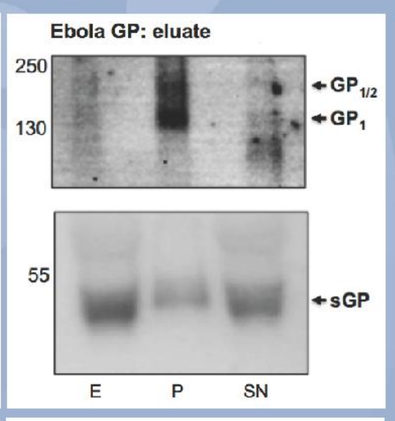


Extracorporeal Treatment
1) multiFiltrate-CiCa®, CiCa-Dialysate K2 *(Fresenius Medical Care, Bad Homburg, Germany (FMC))*
2) CiCa-CVVHDF: postdilution, FMC Ultraflux dialyzer, blood flow 100 ml/min
3) LAPD *(Aethlon Hemopurifier)*: upstream of the dialyzer (EBOV day 13)
4) No adverse event during 6,5 hrs of treatment (no clogging or clotting, no hemolysis, no allergic reaction)
5) qRT-PCRs after flushing and eluting the LAPD: binding of at least 253,160,000 Ebola copies



Ebola glycoprotein (sGP, shed GP and ssGP) **structure** (modified after Cook *et al.* and Dolnik *et al.*)





S: serum P: pelleted virus from serum SN: supernatant after virus peletting	goat anti-EBOV & ZEBOV 3B11mab	E: eluate (α-Methyl-Mannoside, aMM) P: pelleted virus from the eluate SN: supernatant after virus peletting

Ebola glycoproteins (sGP and shed GP) **in serum** (pre/post-LAP) **and in eluate** (PBS, aMM (shown), AVL)

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