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Elimination of Herpes Simplex Virus-2 and Epstein-Barr Virus With Seraph 100 Microbind Affinity Blood Filter and Therapeutic Plasma Exchange: An Explorative Study in a Patient With Acute Liver Failure

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OBJECTIVES: Herpes simplex virus (HSV)-2 is a rare cause of hepatitis that can lead to acute liver failure (ALF) and often death. The earlier the initiation of acyclovir treatment the better the survival. With regard to ALF, controlled randomized data support the use of therapeutic plasma exchange (TPE) both as bridge to recovery or transplantation—possibly by modulating the systemic inflammatory response and by replacing coagulation factors. Seraph 100 Microbind Affinity Blood Filter (Seraph; Ex Thera Medical, Martinez, CA), a novel extracorporeal adsorption device, removes living pathogens by binding to a heparin-coated surface was shown to efficiently clear HSV-2 particles in vitro. Here, we tested the combination of Seraph with TPE to reduce a massive HSV-2 viral load to reach a situation in that liver transplantation would be feasible.

DESIGN: Explorative study.

SETTING: Academic tertiary care transplant center.

PATIENT: Single patient with HSV-2–induced ALF.

INTERVENTIONS: TPE + Seraph 100 Microbind Affinity Blood Filter.

MEASUREMENTS AND MAIN RESULTS: We report Seraph clearance data of HSV-2 and of Epstein-Barr virus (EBV) in vivo as well as total viral elimination by TPE. Genome copies/mL of HSV-2 and EBV in EDTA plasma were measured by polymerase chain reaction every 60 minutes over 6 hours after starting Seraph both systemically and post adsorber. Also, HSV-2 and EBV were quantified before and after TPE and in the removed apheresis plasma. We found a total elimination of $1.81 \times e^{11}$ HSV-2 copies and $2.11 \times e^{6}$ EBV copies with a single TPE (exchange volume of 5L; 1.5× calculated plasma volume). Whole blood clearance of HSV-2 in the first 6 hours of treatment was 6.64 mL/min (4.98–12.92 mL/min). Despite much lower baseline viremia, clearance of EBV was higher 36.62 mL/min (22.67–53.48 mL/min).

CONCLUSIONS: TPE was able to remove circulating HSV-2 copies by 25% and EBV copies by 40% from the blood. On the other hand, clearance of HSV-2 by Seraph was clinically irrelevant, but Seraph seemed to be far more effective of removing EBV, implicating a possible use in EBV-associated pathologies, but this requires further study.

KEY WORDS: absorption; acute liver failure; herpes; viral load

Herpes simplex virus (HSV)–2 is a rare cause of hepatitis that can lead to acute liver failure (ALF) with a high mortality of up to 50–90% (1). Diagnosing this condition is generally difficult and often done only

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postmortem. Early acyclovir therapy is the cornerstone of treatment and is associated with better survival (1). According to the European Association of the Study of the Liver (EASL) guidelines, high-volume therapeutic plasma exchange (TPE) has been graded a 1A recommendation to support ALF patients by removing danger-associated molecular patterns and cytokines along with the simultaneous replacement of missing coagulation factors (2). An explorative case report further highlights the potential use of TPE in patients with HSV-2 hepatitis. However, the effect of TPE on viral load in HSV-associated ALF has not previously been reported (3).

Seraph 100 Microbind Affinity blood filter (Seraph) from Ex Thera Medical, Martinez, CA, is an extracorporeal hemoadsorber that can effectively bind certain pathogens in bloodstream infections. It is used in combination with anti-infective therapy. The technology is based on heparin-coated polyethylene beads that bind and thereby remove microorganisms similar to the interaction with heparin sulfates within the endothelial glycocalyx on the cell surface (4). In in vitro studies, it was demonstrated that a wide range of bacteria can bind to Seraph and that up to 99% pathogen reduction has been observed with a single treatment. Feasibility and safety of Seraph was recently assessed in critically ill COVID-19 patients, where it was generally well tolerated (5) and efficient with regard to severe acute respiratory syndrome coronavirus-2 nucleocapsid protein removal (6). Its effect on HSV-2 in vivo is unknown, but in vitro Seraph showed encouraging results with regard to HSV-2 (7)

We recently treated a 60-year-old female patient with ALF due to fulminant HSV-2 infection. The patient, known for substituted hypothyroidism and colitis ulcerosa treated with alternative medicine for years, presented herself to a primary hospital with perforation of the descending colon and purulent peritonitis. Two days after hemicolectomy and start of antibiotic therapy, immunosuppression with infliximab was initiated. After 2 more days, a fulminant rise of transaminases with normal bilirubin as well as coagulopathy (factor V activity < 10%) was observed, and the patient showed progressive encephalopathy but was still hemodynamically stable. Due to ALF, she was transferred to the transplant center. Therapy with acetylcysteine was started, and a first session of TPE performed. TPE leads to an improvement in coagulation parameters and a reduction of inflammatory markers (C-reactive protein [CRP] from 90 to 31 mg/L, procalcitonin from 6.37 to 4.92 μg/L). With laboratory evidence of a massive viral load of HSV-2 and matching biopsy, 4 days after manifestation of ALF, therapy with acyclovir was initiated, and the second TPE performed. Further on a clinically irrelevant reactivation of Epstein-Barr virus (EBV) was observed and interpreted in the context of immunosuppression. With increasing encephalopathy and rising ammonia under conservative therapy with lactulose and rifaximine, continuous veno-venous hemodialysis (cvvHD) was started. With a factor V activity less than 10% and encephalopathy grade III according to the West Haven classification, the patient fulfilled the Clichy criteria, which are used in Switzerland for transplantation ALF. With regard to transplant evaluation, we came to the conclusion that the persistent massive viral load (so far unresponsive to high-dose acyclovir treatment) was associated with an unacceptable high risk of liver graft infection and consecutive failure. To further support viral clearance in order to reach a situation in which transplantation would be feasible and safe from an infectious disease point of view additionally to TPE and cvvHD, hemoadsorption with Seraph was initiated. Combination of these therapies leads to stable inflammatory markers (CRP 26–31 mg/L, procalcitonin 2.89–2.61 μg/L) and a stabilization of bleeding and hemodynamics. Despite the stabilization, interdisciplinary re-evaluation leads to the consensus that the patient was not a suitable transplant candidate until complete resolution of HSV-2 would be achieved. Unfortunately, she died 5 days later due to perforation of the colon with a consecutive switch to palliative care. Here, we report in vivo clearance of HSV-2 and EBV with Seraph and viral elimination by TPE, respectively.

METHODS

General consent of the patient to use her anonymized data for research was obtained at admission to the university hospital of Zurich (Version 3.0, 01.10.20219). Given that the explorative therapeutic use of Seraph was part of our individualized treatment approach and the existing CE certification of the device, the regional ethics committee has confirmed that no additional approval is required. General treatment in the university hospital followed the EASL guidelines (2), and procedures were

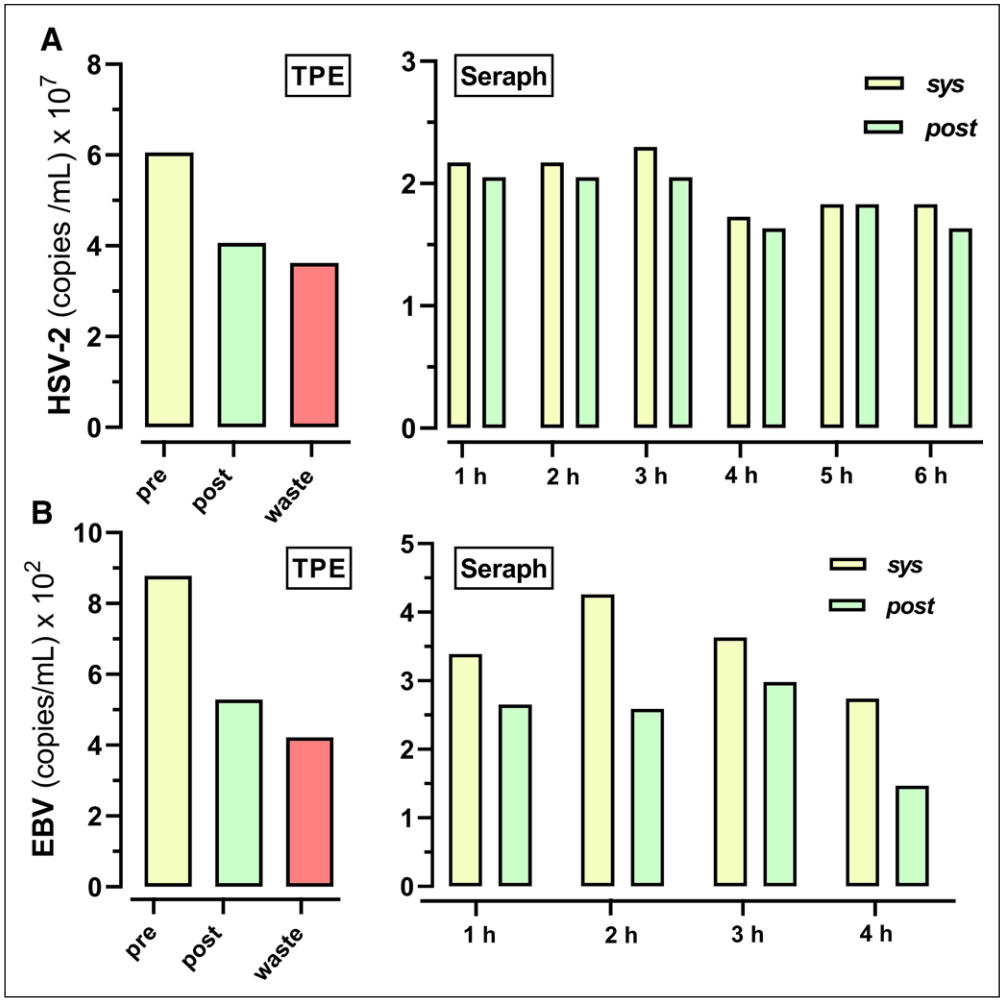

Figure 1. Viral elimination with therapeutic plasma exchange and Seraph at different time points. *Bar graph*s showing herpes simplex virus (HSV)–2 (**A**) and Epstein-Barr virus (EBV) (**B**) copies/mL plasma. Left side visualizes viral quantities in the plasma before (pre) and after (post) therapeutic plasma exchange (TPE) and in the apheresis waste bag. Right side shows viral copies during the first 6 hr auf Seraph treatment both in the systemic circulation (sys) and in the extracorporeal circuit post adsorber (post).

followed in accordance with the ethical standards of the regional committee on human experimentation and with the Helsinki Declaration of 1975.

TPE was started the first day after transfer to the transplant center, and acyclovir was administered IV at a dose of 10 mg/kg/bodyweight with a prolonged interval of 12 hours due to cvvHD as early as the diagnosis of HSV-2 was confirmed by polymerase chain reaction (PCR).

The Seraph was connected in series to our standard hemodialysis circuit (Fresenius, multifiltratePRO) with regional citrate anticoagulation over 22 hours with a blood flow (Q_B) of 120 mL/min. Q_B was chosen lower than usual to reduce citrate load for the patient. TPE was performed according to our in-house standards exchanging 1.5 × plasma volumes with fresh frozen plasma (a total of 5L). Viral load (i.e., genome copies/mL) was estimated according to the cycling time (Ct) of the PCR that was performed in EDTA plasma from samples taken hourly over a 6-hour course after starting the absorption therapy assuming that viral clearance would be maximal during this time frame and before starting another TPE. We collected both systemic, pre-Seraph (C_{in}), and post-Seraph (C_{out}) samples to calculate the HSV-2 and EBV clearances using the measured concentrations as well as the cvvHD Q_B: clearance = $Q_B \times (C_{in}–C_{out})/C_{in}$. Median clearance and interquartile range (IQR) are provided. Additionally, systemic plasma samples were collected before and after TPE, and an apheresis sample from the waste bag was taken at the end of the TPE.

RESULTS

Before initiation of TPE, a very high HSV-2 viral load of 60.54 e^6 copies/mL was measured with a clinically relevant reduction to 40.64 × e^6 copies/mL at the end of TPE. In the removed apheresis waste plasma, the viral load was 36.26 × e^6 copies/mL. Exchanging 5 L of plasma in total consequently corresponds to 1.81 × e^{11} removed HSV-2 copies (**Fig. 1*A***). Regarding EBV, we saw a reduction from 878 to 529 copies/mL. With 422 copies/mL measured in the apheresis waste plasma, a total of 2.11 × e^6 copies were removed (**Fig. 1*B***).

Regarding HSV-2 before start of Seraph (time point [t] 0) the next morning, there were still 19.38 × e^6

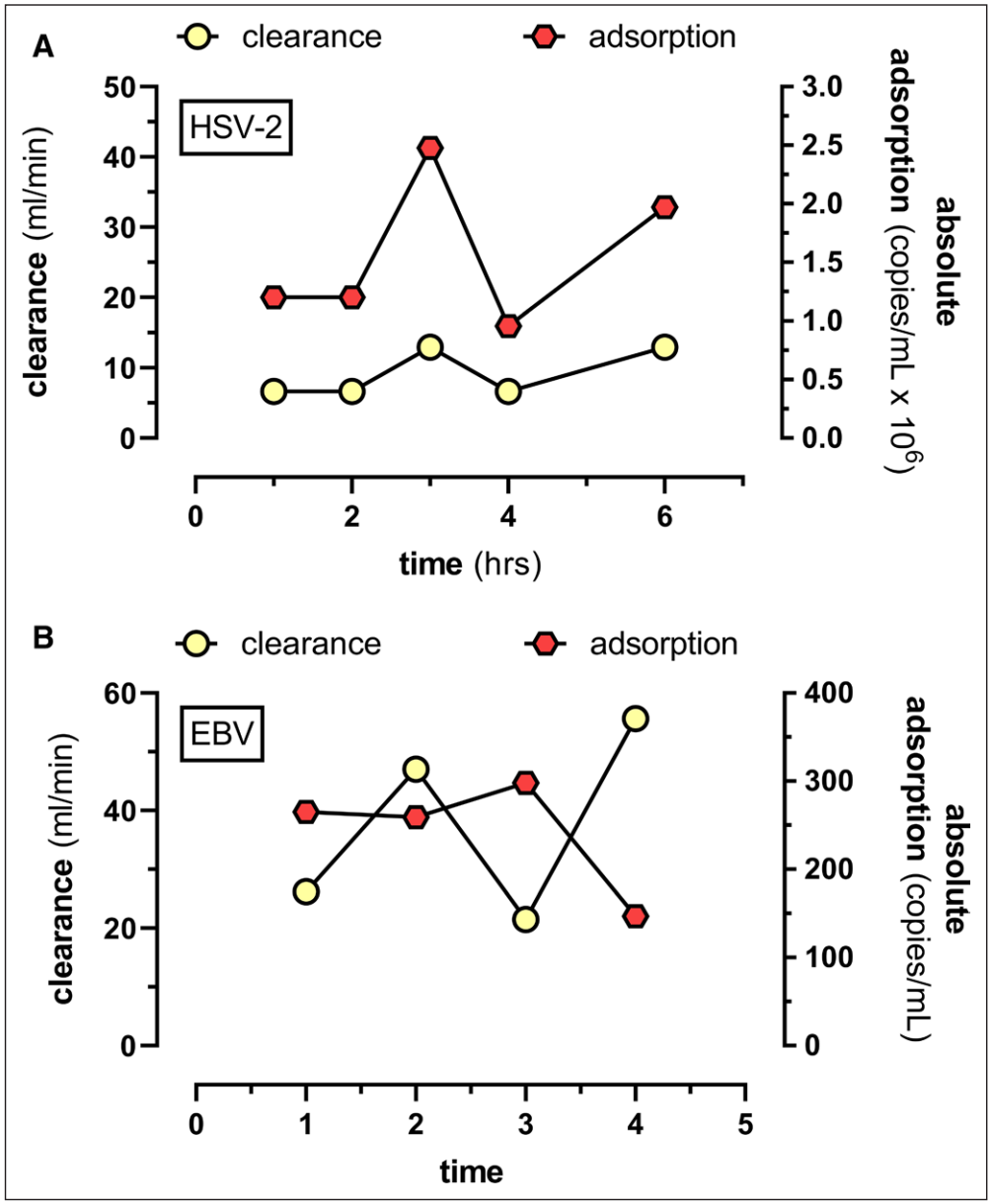

Figure 2. Clearance and absolute adsorption of herpes simplex virus (HSV)–2 and Epstein-Barr virus (EBV) with Seraph at different time points. Left axis shows Seraph clearances of (**A**) HSV-2 and (**B**) EBV in the first 6 hr of treatment (*yellow circles*). Right axis shows total absolute adsorption) in *red circles*.

copies/mL in the blood detectable. After 1 hour (t1), we found systemic 21.72 × e^6 copies/mL and C_{out} 20.52 × e^6 copies/mL resulting in a calculated clearance of 6.64 mL/min (**Fig. 2*A***). At the timepoint t 5, there was no difference in the Ct-value C_{in} and C_{out}, leading to a theoretical clearance of 0 mL/min. Median calculated clearance for HSV (CL_{HSV-2}) was 6.64 mL/min (4.98–12.92 mL/min).

Given that the patient also had a (clinically irrelevant) coinfection with EBV, we additionally quantified the EBV load and measured clearances accordingly. **Figure 2*B*** shows the absolute adsorption (calculated of C_{in} and C_{out}) as well as the calculated clearance for EBV (CL_{EBV}). Median CL_{EBV} was with 36.62 mL/min (22.67–53.48 mL/min) higher than the CL_{HSV-2}.

DISCUSSION

Regarding the high mortality of HSV-2 hepatitis and acyclovir being the only specific therapeutic option that has shown to reduce mortality if given early, an alternate approach to reduce viral load would be highly desirable. This is of particular importance in the context of an urgent liver transplantation when additional immunosuppression is required but the virus is not yet cleared. Together with a per se more vulnerable graft compared with a native liver, this might be associated with a substantial risk of HSV-2 de novo infection and thereby early failure of the graft. Along the same lines, there are reports of donor-derived HSV infections in solid organ transplantation, where a therapeutic viral load reduction could also be of potential benefit (8).

Obviously, extracorporeal adsorption comes with a potential risk of removal of antimicrobial substances during the procedures. However, in vitro data have shown no clinically significant elimination of acyclovir by hemadsorption with Seraph (9) or TPE (10).

In contrast to the encouraging in vitro data, we observed a low CL_{HSV-2} with Seraph of only 6.64 mL/

min despite its very high abundance in the circulation. Interestingly, CL_{EBV} (a clinically irrelevant coinfection in this particular patient) was much better (i.e. 36.6 mL/min) despite a dramatically lower viral load to begin with. We can only speculate why the elimination of the two *Herpesviridae* HSV-2 and EBV by Seraph was so different. One possibility is the difference in structure that might have influenced the interaction with the immobilized heparin in Seraph (11). EBV has a strong binding to heparinsulfate rich proteoglycans making it potentially more accessible for binding to the Seraph surface.

Given that TPE has become standard of care for patients suffering from ALF in many transplant centers, it was obvious to analyze if the simple exchange of plasma was also sufficient to remove virus particles. Indeed, we found a rather impressive reduction of HSV-2 viral load that even exceeded the effect of Seraph. Therefore, we assume that adding Seraph to the conventional therapy of ALF caused by HSV-2 appeared to have no major additional impact on viral elimination. The interpretability of the data is certainly limited with only one patient studied and without a control group. Furthermore, the Q_B rate was, although constant, with 120 mL/min relatively low due to the risk of citrate accumulation. With respect to the convincing removal of HSV-2 by TPE, it is important to acknowledge that our data are exclusively derived from plasma samples before and after a treatment procedure. We did not perform serial quantification of HSV-2 in the course after TPE making it speculative to consider redistributive effects of HSV-2 from third spaces after the TPE was stopped. Further on although Seraph was included in the cvvHD circuit for 22 hours, we report only data from the first 6 hours were no TPE in parallel was performed and redistribution effect was assumed to be negligible.

CONCLUSIONS

In contrast to the encouraging in vitro data, we observed a relatively low clearance of HSV-2 in vivo using Seraph. Adding it to conventional therapy in ALF due to HSV-2 seemed to have no relevant impact on viral elimination. In contrast, a good clearance of EBV by Seraph was observed despite a lower viral load. The fact that no specific EBV treatment is available makes this entity an interesting target candidate for Seraph that deserves further evaluation.

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Drs. Andermatt, Ganter, N.J. Mueller, Muellhaupt, and David treated the patient and collected the data. Drs. Andermatt and David wrote article. Dr. Bloemberg performed the viral analysis. Dr. Kielstein gave his expert opinion on Seraph and Dr. A. Mueller hers on therapeutic plasma exchange. All authors reviewed the article.

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Next of kin gave their permission for publication of this explorative study.

Authors can confirm that all relevant data are included in the article. The corresponding author may provide specified analyses or fully de-identified parts of the dataset upon reasonable request.

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