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Clinical paper

Elimination of glutamate using CRRT for 72 h in patients with post-cardiac arrest syndrome: A randomized clinical pilot trial

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Abstract

Aim: Glutamine and glutamate are major mediators of secondary brain cell death during post-cardiac arrest syndrome. As there is an equilibrium between brain tissue and plasma concentrations of glutamine and glutamate, their elimination from systemic circulation by extracorporeal blood purification may ultimately lead to reduced secondary cell death in the brain. We hypothesized that systemic glutamine and glutamate can be significantly reduced by continuous venovenous hemodiafiltration (CVVHDF).

Methods: This was a prospective, randomized clinical trial in post cardiac-arrest survivors evaluating standard of care or additional CVVHDF over 72 h immediately after admission. Glutamine and glutamate plasma concentrations were analyzed at eight time points in both groups. Primary endpoint was reduction of glutamine and glutamate plasma concentrations. The trial has been registered at clinical trial.gov (NCT02963298).

Results: In total, 41 patients were randomized over a period of 12 months (control n = 21, CVVHDF n = 20). The primary aim reduction of glutamine and glutamate plasma concentrations by CVVHDF, was not achieved; both groups-maintained concentrations within a normal range over the study period (glutamate: 4.7–11.1 mg/dL; glutamine: 0.2–3.7 mg/dL). However, post-filter concentrations of glutamine and glutamate in CRRT patients were significantly decreased as compared to pre-filter concentrations (glutamate: pre-filter median 8.85 mg/dL IQR 7.1–9.6; post-filter 0.95 mg/dL IQR 0.5–2; $p < 0.001$; glutamine: pre-filter 0.7 mg/dL IQR 0.6–1; post-filter 0.2 mg/dL IQR 0–0.2; $p < 0.001$).

Conclusion: In this trial, CVVHDF was not able to statistically significantly lower systemic plasma glutamine and glutamate levels. Post-cardiac arrest patients had plasma glutamine and glutamate levels within the normal range.

Keywords: Post-cardiac arrest syndrome, Glutamate, Continuous renal replacement therapy

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Introduction

The severity of post-resuscitation syndrome is a major factor determining neurological outcome. Current guidelines recommend a combination of several treatments to ameliorate post-resuscitation syndrome, and therefore improve overall and neurological outcomes.[1] It is well known that secondary cell death following return of spontaneous circulation can aggravate hypoxic brain damage[2]. Glutamate, an excitatory neurotransmitter, is an important excitotoxic mediator by inducing cellular calcium overload leading to apoptosis.[3,4] Glutamine can be generated in two enzymatic steps from glutamate, with equilibrium of both molecules in the body; therefore, reduction in plasma glutamine leads to decreased plasma glutamate. Additionally, as glutamate follows a gradient at the blood-brain barrier, its brain concentration can be decreased by lowering its plasma concentration.[5–7] A reduction of glutamate by using different scavengers in rat models resulted in decreased neuronal injury.[8–11] To date, there are no clinical data in post-cardiac arrest patients to indicate whether a systemic reduction of plasma glutamate and glutamine concentrations by CVVHDF is feasible and safe, nor whether decreased concentrations ultimately result in improved neurological outcomes. This clinical feasibility study therefore aimed to evaluate the plasma concentrations of glutamate and glutamine over the first 72 h following hospital admission in patients randomized to either standard therapy or standard therapy plus CVVHDF.

Methods

This study was approved by the local ethics committee of the Charité-Universitätsmedizin Berlin (EA4/048/16) and registered at clinical trial.gov (NCT02963298). Written informed consent for trial participation was obtained by a legal custodian as all patients were undergoing sedation at time of inclusion. The design was a prospective, randomized open-label trial. Randomization was performed by use of a sealed envelope system following application of inclusion (out-of-hospital cardiac arrest, age $\geq$18 years) and exclusion (in-hospital cardiac arrest, hemodynamically instable) criteria during hospital admission. Standard therapy was administered according current guidelines for post-cardiac arrest patients, including maintenance of body temperature at a target of 33 °C over 24 h, followed by a rewarming phase of 16 h (0.25 °C/h). Patients randomized to the intervention group additionally received continuous venovenous hemodiafiltration (CVVHDF), a continuous renal replacement therapy (CRRT), for the first 72 h following admission. For CVVHDF, the multiFiltrate (Fresenius Medical Care, Germany) was used at a blood flow rate of 100 mL/h and total effluent fluid flow rate of 3000 mL/h, with replacement fluid administered at 1000 mL/h. The local standard protocol for citrate anticoagulation was used in all patients. Blood samples were taken as indicated in Table 1. All samples were directly centrifuged (4000 RPM for U/min/10 min); plasma samples were stored immediately at −80 °C. High-pressure liquid chromatography mass-spectrometry (HPLC-MS) was used to analyze plasma samples

Table 1 – Baseline characteristics of study cohort; CRRT continuous renal replacement therapy, tROSC time to ROSC, VF ventricular fibrillation, PEA pulseless electrical activity, AMI acute myocardial infarction, NSE neuron specific enolase, APACHE Acute Physiology And Chronic Health Evaluation, CPC cerebral performance category, WLST withdrawal of life support treatment, MOV multi-organ failure.

Variables	Control N = 21	CRRT N = 20
Age (years; median [IQR])	59.00 [54.50, 69.00]	67.5 [56.00, 74.00]
Epinephrin (mg; median [IQR])	2.00 [1.00, 5.00]	3.50 [2.00, 5.00]
tROSC (min; median [IQR])	15.00 [12.00, 25.00]	20.00 [9.50, 25.50]
First rhythm (%)		
VF	11 (52.4)	9 (45.0)
Asystole	5 (23.8)	5 (25.0)
PEA	5 (23.8)	6 (30.0)
Gender male/female (%)	17/4 (81.0/19.0)	13/7 (65.0/35.0)
Diagnosis		
AMI	11 (52.4)	7 (35.0)
Primary arrhythmia	2 (9.5)	2 (10.0)
Respiratory cause	5 (23.8)	6 (30.0)
Cardiogenic shock	0 (0.0)	2 (10.0)
Unknown	3 (14.3)	3 (15.0)
Respirator time (h) (median [IQR])	176.00 [127.00, 328.00]	281.50 [69.50,455.25]
NSE day 3 (µg/L; median [IQR])	62.00 [27.00, 176.00]	28.80 [13.60, 161.15]
APACHE (median [IQR])	36.00 [31.00, 38.00]	38.00 [34.75, 39.50]
CPC (%)		
1	4 (19.0)	2 (10.0)
2	4 (19.0)	5 (25.0)
3	2 (9.5)	0 (0.0)
4	1 (4.8)	1 (5.0)
5	10 (47.6)	12 (60.0)
Cause of death		
WLST	6	5
MOV	2	5
Other	2	2

at an external medical laboratory (Medizinisches Labor Bremen) after the end of the inclusion phase. Laboratory reference ranges for glutamate and glutamine were 4.7–11.1 mg/dL and 0.2–3.7 mg/dL, respectively. The laboratory was blinded toward group allocation, clinical course, and clinical outcome of patients.

The primary outcomes measured were changes in glutamate and glutamine plasma concentrations due to CVVHDF. Secondary parameters evaluated were neurological outcome: neuron-specific enolase (NSE), evoked potentials (SSEP) and Pittsburg Cerebral Performance Category (CPC) at discharge. Neuro-prognostication was performed by repetitive neurological evaluation, biomarker testing (NSE serum concentration 72 h after CA), neurophysiological testing (SSEP, EEG) between day 2–7 and brain imaging (CT/GWR) as appropriate according to our local standard, in agreement with national and international guidelines. Recommendation of withdrawal of ICU treatment was based on the results of this multimodal approach and made after 5–7 days after cardiac arrest the earliest in the majority of the patients.

Statistical analysis

Results are reported as proportions (%) or median values, including the interquartile range (IQR). As appropriate, tests for statistical significance were performed using a Mann–Whitney U test or Fisher's exact test for independent measurements, or a paired Wilcoxon signed-rank test for dependent measurements. To analyze repeated measurements for the time points A, C, D, E, F, G and H over the time course, a linear mixed model was used. A two-tailed p-value less than 0.05 was considered statistically significant. All statistical analyses were performed with R (v. 3.5.0 R Foundation, Vienna, Austria) using the packages lme4 and nlme.

Results

In total, 165 patients were admitted to our ICU following cardiac arrest between August 2016 and August 2017;41 were included in the trial (Fig. 1). There were no significant differences in baseline characteristics between groups in the study population (Table 1).

Glutamate and glutamine plasma concentrations

Detailed glutamate and glutamine plasma concentrations between the experimental groups are given in Table 2 and Figs. 2 and 3. There were no differences between experimental groups when plasma concentration was reported as a change from baseline concentration (linear mixed model; glutamate $p = 0.85$, glutamine $p = 0.16$)

Parameters of neurological prognosis were also analyzed. NSE after 72 h was not significantly different in the intervention as compared to the control group (NSE median/IQR, CVVHDF 28.80 [13.60, 161.15] μg/L, control 62.00 [27.00, 176.00]; $p = 0.242$).

Following the local standard protocol, 14/20 patients (CRRT group) and 16/21 (control) underwent measurement of SSEP by stimulation of the Median Nerve. Results were categorized into normal, no cortical potential and low cortical potential. Eight patients in the CRRT group had normal, 5 low cortical and 1 patient no cortical potential. Ten patients in the control group had normal, 1 low cortical and 5 patients no cortical potential. The clinical neurological status was assessed by the CPC score; 7/20 (35%) in the CRRT group and 8/21 (38%) in the control group had a good neurological outcome (CPC 1-2). There was no significant difference in outcome between the groups ($p = 0.568$). No severe adverse events were reported in either group.

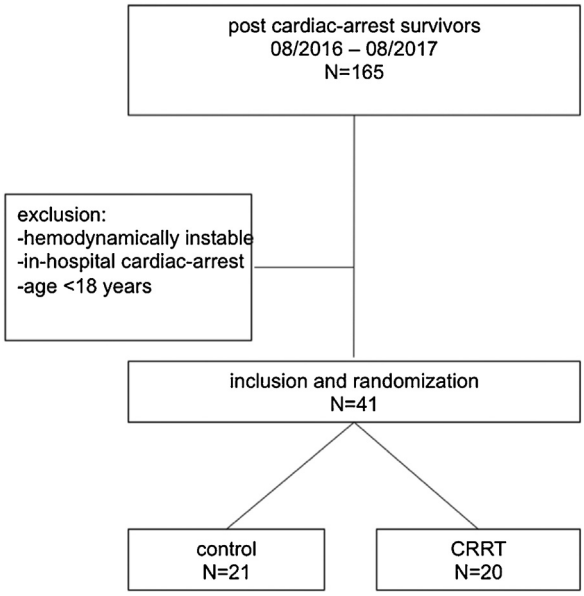

Fig. 1 – Study flowchart; MAP = mean arterial pressure, IHCA = in-hospital cardiac-arrest, CRRT = continuous renal replacement therapy in the intervention group.

Post-filter concentration

In the CRRT group, a sample was taken post-filter (time point B) following the start of CRRT. In comparison to the initial plasma concentration (pre-filter) the decrease was statistically significant for glutamate (paired Wilcoxon signed-rank test; pre-filter median 8.85 mg/dL IQR 7.1–9.6; post-filter 0.95 mg/dL IQR 0.5–2; $p < 0.001$) as well as for glutamine (pre-filter median 0.7 mg/dL IQR 0.6–1; post-filter 0.2 mg/dL IQR 0–0.2; $p < 0.001$).

Discussion

The main results of this monocentric, randomized pilot trial were (1) patients after cardiac arrest had plasma glutamate and glutamine levels that were within normal limits, (2) there was no significant decrease in plasma glutamate concentration following CRRT, (3) there was no significant difference between randomized groups in terms of neurological prognostic parameters and neurological outcome, and (4) CRRT was used safely in patients after cardiac arrest and resuscitation for 72 h. Notably, in this proof of concept pilot study, post-filter glutamate and glutamine concentrations decreased significantly following CRRT.

To our knowledge, this is the first pilot study using CRRT to target cerebral glutamate clearance by aiming to lower systemic glutamate and glutamine levels. Surprisingly, no difference in the glutamate and glutamine concentration between the control group and the CRRT group was found, though post-filter concentrations decreased significantly. However, corresponding glutamate brain levels were

Table 2 – Laboratory results of glutamate and glutamine measurement at all timepoints; A (admission), C (1 h after admission), D (2 h after admission), E (4 h after admission), F (24 h after admission), G (48 h after admission) and H (72 h after admission). Time point B (admission post-filter) is not shown in the table. SD standard deviation, SE standard error, CRRT continuous renal replacement therapy.

Group	Measurement	n	Mean glutamine (mg/dL)	SD	SE
CRRT	A	20	0.840	0.310	0.069
CRRT	C	18	0.917	0.601	0.142
CRRT	D	16	0.863	0.379	0.095
CRRT	E	16	0.856	0.392	0.098
CRRT	F	16	0.913	0.266	0.066
CRRT	G	14	0.836	0.273	0.073
CRRT	H	13	0.785	0.267	0.074
Control	A	21	1.100	0.593	0.129
Control	C	21	1.162	0.663	0.145
Control	D	20	1.265	0.903	0.202
Control	E	20	1.180	0.797	0.178
Control	F	20	0.970	0.387	0.086
Control	G	19	1.179	0.421	0.097
Control	H	17	0.988	0.209	0.051
Group	Measurement	n	Mean glutamate (mg/dL)	SD	SE
CRRT	A	20	8.575	1.913	0.428
CRRT	C	18	8.139	1.935	0.456
CRRT	D	16	7.663	1.731	0.433
CRRT	E	16	7.613	2.354	0.589
CRRT	F	16	8.913	1.803	0.451
CRRT	G	14	8.536	2.122	0.567
CRRT	H	13	8.369	3.420	0.949
Control	A	21	7.776	1.898	0.414
Control	C	21	7.605	2.211	0.483
Control	D	20	7.610	2.647	0.592
Control	E	20	7.420	2.653	0.593
Control	F	20	8.095	5.109	1.142
Control	G	19	7.189	3.341	0.766
Control	H	17	6.365	1.759	0.427

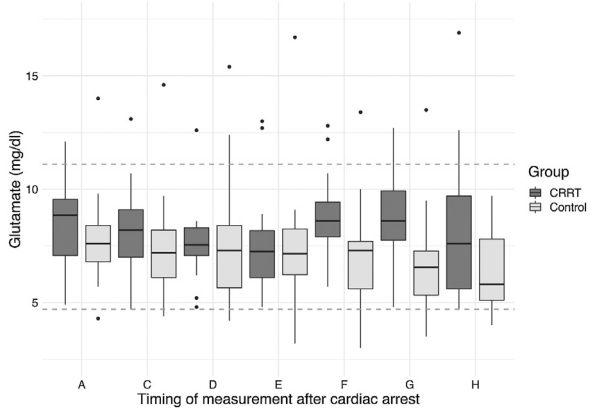

Fig. 2 – Glutamate levels at time points A (admission), C (1 h after admission), D (2 h after admission), E (4 h after admission), F (24 h after admission), G (48 h after admission) and H (72 h after admission) in both groups, area between dotted lines indicates normal range of glutamate (4.7–11.1 mg/dL), black dots indicate outliers; CRRT = continuous renal replacement therapy in the intervention group. Time point B (admission post-filter) is not shown in the figure.

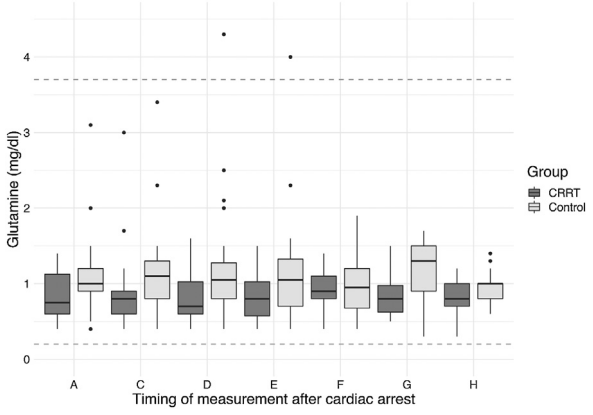

Fig. 3 – Glutamine levels at time points A (admission), C (1 h after admission), D (2 h after admission), E (4 h after admission), F (24 h after admission), G (48 h after admission) and H (72 h after admission) in both groups, area between dotted lines indicates normal range of glutamine (0.2–3.7 mg/dL), black dots indicate outliers; CRRT = continuous renal replacement therapy in the intervention group. Time point B (admission post-filter) is not shown in the figure.

not obtained and therefore it remains unknown whether CRRT had an effect towards the gradient at the blood-brain-barrier. It is possible that the effluent fluid flow rate of 3000 mL/h was insufficient to elicit a statistically significant decrease in plasma concentrations. Higher clearances might have been achieved with intermittent hemodialysis (iHD) using higher blood and dialysate flow rates. A CRRT procedure was used in this study to enable constant glutamate elimination from the plasma over time. Furthermore, CRRT has the lowest expected complication rate with respect to hemodynamic stability and patient safety in this patient group after resuscitation.[12]

Rogachev et al. recently demonstrated a decrease in blood glutamate levels within the first three hours after initiation of iHD in stage V renal disease-affected patients receiving iHD on a regular basis.[13] These authors also evaluated glutamate elimination in patients receiving peritoneal dialysis (PD) and found an initial decrease of around 20% within the first 60 min after start of PD, followed by a slow return to baseline blood glutamate levels.[14] As a possible explanation of the observed rebound of glutamate plasma concentration the authors postulated that this may represent a reaction of neurons in response to disequilibrium of urea in the cerebrospinal fluid and blood: during PD and iHD, urea is removed from the plasma at a rate that exceeds removal from the tissues, which induces an influx of water into the extracellular space. Neurons are hypothesized to counteract this osmotic gradient by increasing cellular export of glutamate into the cerebrospinal fluid and plasma. Whilst iHD may provide the highest clearance rates of small solutes including glutamate and glutamine, its use is limited in hemodynamically unstable patients as well as in patients after rescue percutaneous coronary intervention. Conversely, acute PD may help to preserve hemodynamic stability in critically ill patients, however, the clearance rates for small solutes that can be achieved with typical treatment protocols are typically lower than those feasible with CVVHDF.

In general, glutamate levels in muscle and liver tissue are 100 times higher than in the blood.[14] These depots offer a high amount of substrate to compensate and balance blood glutamate levels, and may explain the short-lived effects of external reduction of plasma glutamate concentration.[15] Brotfain et al. reported a small series of 10 patients undergoing hemofiltration (HF) with acute renal failure due to sepsis or trauma.[16] Glutamate blood levels were slightly above the normal range and decreased over the first 12 h of treatment (20% reduction from baseline). Calculating the highest percent of reduction from baseline (sample A) in our cohort, the reduction in the CRRT group was 11.25% (sample A to lowest level at sample C) and in the control group 18.15% (sample A to lowest sample H), indicating a dynamic range independent from the treatment. It remains speculative whether the duration of ischemia (time to return of spontaneous circulation; tROSC) in patients in our study was too brief, but the observed span (9–25 min) is in line with other published cohorts.[17] Furthermore, tROSC was not significantly different between our randomized groups.

On the basis of our current findings a potential next step should be directed at the optimization of intensity and modality of extracorporeal treatment, thereby ideally measuring glutamate levels simultaneously in plasma and cerebrospinal fluid. This would help to determine if indeed a concentration gradient can be achieved with different dialysis strategies.

Limitations

First, this randomized pilot trial is small and has therefore limitations in interpretation. Second, the brain tissue concentration of glutamate and glutamine was not known in the investigated group and our findings of glutamate and glutamine within the normal range could be a limitation as well. Third, randomization was performed by a sealed envelope system instead of more advanced techniques such as central phone or computer methods. Fourth, post cardiac arrest patients are known to be heterogenous, reducing the significance to be drawn from small cohorts in general. *Fifth, hemodynamically instable patients were excluded due to safety reasons as vasopressor support may have been increased during CRRT. However, we acknowledge that we may have missed patients with high glutamate concentrations as one can hypothesize, that a higher need for vasopressors may indicate a more severe myocardial dysfunction following longer no- or low flow time.* This study was investigator initiated but supported with a research grant of 198.000€ provided by Fresenius Medical Care Deutschland GmbH. The company had no influence in study design, realization of the trial or data analysis.

Conclusion

This randomized clinical pilot trial showed that post-cardiac arrest patients have normal plasma concentrations of glutamate and glutamine, both initially on presentation and over the first 72 h following ROSC. In this study, there was no significant effect of CRRT on plasma levels. Neurological outcome was equally distributed among the two experimental groups; however, the sample size was small and not adequately powered for outcome analysis.

Follow-up studies are needed to determine if peripheral elimination of glutamate is feasible with other, more intensive, dialysis strategies and, even more importantly, whether a concentration gradient between brain an systemic circulation can be induced by extracorporeal glutamate elimination in post-cardiac arrest patients.

Source of funding

The study was supported by a grant of 198.000€ by Fresenius Medical Care Deutschland GmbH.

Conflict of interest

CS has received honoraria as a speaker and consultant from BD BARD GmbH and Sedana Medical and as speaker from Zoll GmbH and Philips. CM and SS are full-time employees of Fresenius Medical Care Deutschland GmbH. AK has no conflict of interest related to this publication but independent from this publication and the presented topic, however, AK has a financial relationship to Pfizer Deutschland GmbH (Linkstraße 10, 10785 Berlin, Germany). CL has received remuneration for presentations and travel costs from BD BARD and has received honoraria from Edwards Lifesicences GmbH (Edisonstraße 6, 85716 Unterschleißheim, Germany) for work in a Critical Event Committee. JN received honoraria and travel costs for presentations from BD BARD, Fresenius Medical Care Deutschland GmbH and Xenios AG. AJ has received honoraria as a speaker and consultant for Fresenius Medical Care Deutschland GmbH. PE, ALM and TS report no conflicts of interest.

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