

Randomized, Placebo-controlled, Double-blind, Pilot Trial to Investigate Safety and Efficacy of Cerebrolysin in Patients with Aneurysmal Subarachnoid Hemorrhage

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Abstract

Background

There are limited treatment options for aneurysmal subarachnoid hemorrhage (SAH)-induced delayed cerebral ischemia, a major determinant of mortality and morbidity. Cerebrolysin, a brain-specific pleiotropic neuroprotective agent, has been suggested to improve functional outcomes in ischemic stroke. We investigated the efficacy, safety and feasibility of Cerebrolysin for conferring such benefits in SAH patients.

Methods

This was a prospective, randomized, double-blind, placebo-controlled, single-center, parallel-group pilot study. 50 patients received either daily Cerebrolysin (30ml/day) or a placebo (saline) for 14 days (25 patients per study group). The primary endpoint was a favorable Extended Glasgow Outcome Score (GOSE) of 5 to 8 (moderate disability to good recovery) at six-months. Secondary endpoints included the occurrence of adverse effects, six-month mortality, the occurrence of cerebral vasospasm, delayed cerebral ischemia and infarction.

Results

No severe adverse effects or mortality attributable to Cerebrolysin were observed. No significant difference was detected in the proportion of patients with favorable six-month GOSE in either study group (odds ratio (OR): 0.53; 95% confidence interval (CI): 0.43-5.17). Secondary functional outcome measures for good six-month recovery indicated by a modified Rankin Scale of 0 to 2 (OR: 0.53; 95% CI 0.43-5.17) and a Barthel Index of 70 or more (OR: 0.52; 95% CI: 0.29-12.72) were also similar for both groups. There was a significantly lower risk of three- and six-month mortality for patients that received Cerebrolysin (OR: 0.46; 95% CI: 0.33-0.63). There were no deaths in the Cerebrolysin group, but the mortality rate for the control group was 16% (4/25). The commonest cause of death was due to delayed cerebral ischemia. There were no differences in the overall incidence of delayed cerebral ischemia (p-value: 0.78), cerebral vasospasm (p-value: 0.16) and infarction (p-value: 0.77) between the two groups.

Conclusions

Use of Cerebrolysin in addition to standard-of-care management of aneurysmal SAH is safe, well tolerated and feasible. Results suggest a benefit in reducing three- and six-month mortality. Due to the exploratory nature of this study and its small sample size, these findings should be confirmed in a larger-scale clinical trial.

Background

Aneurysmal subarachnoid hemorrhage (SAH) accounts for less than 5% of all stroke, but given the relatively young age of afflicted patients and the severity of brain insult, it is disproportionately

responsible for 27% of all stroke-related potential years of life lost before the age of 65.[1] Advances in intracranial aneurysm treatment and neurocritical care have substantially improved outcomes, but delayed cerebral ischemia (DCI) continues to be a management challenge.[2–4] DCI is a major determinant of mortality, accounting for up to 50% of all SAH-related deaths, and morbidity in patients that survive the initial hemorrhage.[5, 6] It occurs in 20–40% of patients, but in spite of its recognition as an important complication of SAH, the exact pathophysiology remains to be elucidated.[7] DCI is postulated to be a multifactorial process involving blood-brain barrier disruption, arteriolar vasospasm, microthrombosis, loss of cerebrovascular autoregulation, cortical spreading depolarizations and the triggering of inflammatory cascades.[8] Since it usually occurs between four and 10 days after SAH, there exists a potential therapeutic window that has been subject to intensified research.[5] But to-date clinical trials investigating endothelin receptor antagonists such as clazosentan, lipid peroxidation inhibitors such as tirilazad, aspirin, statins, magnesium, hypertensive therapy, and transluminal balloon angioplasty have generally been unsuccessful.[9–16] The only consistently proven treatment for DCI that can reduce poor clinical outcomes is Nimodipine, a calcium channel blocker, that was identified 30 years ago by Pickard et al and has since been verified by several subsequent trials.[2, 17]

Cerebrolysin (EVER Neuro Pharma GmbH, Unterach, Austria) is an intravenously (IV) administered preparation consisting of a mixture of low-molecular weight neuropeptides of less than 10 kDa (80%) and free amino acids (20%) derived from porcine brain tissue. By addressing multiple targets along the pathophysiological cascade of neuronal cell death, Cerebrolysin has been shown to have neuroprotective properties by promoting anti-apoptosis, counteracting glutamate excitotoxicity, reducing free oxygen radical concentrations, microglial activation and modulating the neuro-inflammatory response in both in vitro and murine ischemic stroke models.[18–25] A multicenter randomized placebo-controlled (RCT) phase III clinical trial that evaluated the therapeutic effects of Cerebrolysin in ischemic stroke patients demonstrated improved three-month functional outcomes.[26] Other RCTs have also supported Cerebrolysin's therapeutic potential for ischemic stroke, enhancing neurocognitive performance, reducing the pulsatility index of the middle cerebral artery (MCA) by transcranial Doppler ultrasound (TCD) and infarct volume by magnetic resonance imaging (MRI).[27–33]

For this prospective randomized, placebo-controlled, double-blind pilot study, we aimed to investigate the effects, safety and feasibility of early Cerebrolysin treatment for patients with aneurysmal SAH.

Methods

This was an investigator-initiated, single-center randomized, placebo-controlled, double-blind, 1:1 parallel-group phase IIa pilot trial that investigated the effects of Cerebrolysin for consecutive adult patients diagnosed with aneurysmal SAH from 1 February 2014 to 30 June 2018. Clinical research ethics committee approval was obtained (Institutional Review Board number: KW/FR-13-006 (61 – 04)). The trial was registered with ClinicalTrials.gov (NCT01787123) and was conducted according to the Declaration of Helsinki and Good Clinical Practice. All subjects or their legal representatives provided written informed consent.

Inclusion and Exclusion Criteria

Patients that fulfilled the study criteria were enrolled by the study investigators. The inclusion criteria were: age 18 to 80 years-old; of Chinese descent; a radiological diagnosis of aneurysmal SAH and that the patient could be randomized within 96 hours of ictus, i.e. before the onset of DCI. Exclusion criteria were: when an unsalvageable disease course was anticipated (death within 48 hours of admission or post-resuscitation signs of central or uncal herniation); pre-existing neurological or psychiatric disorders, including stroke, epilepsy and dementia; a pre-SAH disability (i.e. a modified Rankin score (mRS) of ≥ 2); major cardiac, pulmonary, hepatic and renal disease (i.e. creatinine concentration of $> 200 \mu\text{mol/L}$); terminal medical illness with a life-expectancy of less than one year; an existing diagnosis of DCI or vasospasm; an active history of alcohol or illicit drug dependency; previous history of Cerebrolysin exposure; any contraindication for Cerebrolysin administration, including pregnancy, lactation and allergies to its components; or concurrent participation in another clinical trial.

Subject Management, Randomization and Blinding

After establishing the diagnosis of a ruptured intracranial aneurysm by either computed (CT) tomography angiography or catheter angiography, patients were randomly assigned to receive either intravenous Cerebrolysin (intervention group) or normal saline infusions (placebo group). In prospective studies focused on ischemic stroke, Cerebrolysin dosages varied from 10 to 50 ml per day.[26–28, 30, 33] According to two larger Phase III RCTs, a daily dose of 30 ml Cerebrolysin was administered for a duration of 10 to 21 days.[26, 27] It was decided to adopt a similar daily dose for 14 days in order to cover the four to 10-day period where cerebral vasospasm most frequently occurs after SAH.[34] Subjects in the intervention group received 30 ml of IV Cerebrolysin per day for 14 days. Eight-hourly 30-minute IV infusions consisting of 10 ml of Cerebrolysin diluted with saline to a total volume of 100 ml was given. Subjects in the control group received 100 ml of saline every eight hours for the same period. Allocation was performed according to a predefined block randomization plan generated by the Statistical Package for the Social Sciences version 22.0 (SPSS Inc, Chicago, IL, US). A block size of 10 was used and group allocation within each block was conducted with a 1:1 ratio. Assignment instructions were sealed in envelopes and opened after subject recruitment. Patients and study outcome assessors were blinded to group identity while clinicians directly involved in their medical management were aware. Since Cerebrolysin carries a yellow tint, infusion bags were wrapped in opaque plastic and amber-coloured intravenous tubing were utilized for both study groups in order to mask the infusate administered.

All recruited subjects were treated according to the latest 2012 American Heart Association (AHA)/ American Stroke Association (ASA) Guidelines for the Management of Aneurysmal SAH.[35] In the initial phase all patients were treated at the neurocritical care unit with adherence to Class I and IIa recommendations for the management of DCI: four-hourly doses of Nimodipine 60 mg was administered for 21-days, euvolemia was maintained, induction of hypertension was performed if DCI was diagnosed and intra-arterial vasodilator therapy was performed in patients with symptomatic cerebral vasospasm

unresponsive to hypertensive therapy.[35] When considered fit for post-stroke rehabilitation, all subjects were enrolled into a minimum two-week standardized early inpatient mobilisation physiotherapy program and occupational therapy for basic activities of daily living.

Data Collection and Study Endpoint Assessment

Data from clinical records, operation notes, medication dispensing records, laboratory and radiological investigations were collected by an independent neurosurgeon without knowledge of the subject group assignment. The clinical severity of SAH was classified according to the modified World Federation of Neurosurgical Societies (WFNS) grading scale.[36] Good-grade WFNS was defined as grade I or II, i.e. patients presenting with a GCS of 14 or 15. The Acute Physiology and Chronic Health Evaluation (APACHE II) score, an independent predictor for in-hospital mortality for SAH patients, was also calculated. [37] The Charlson Comorbidity Index (CCI), a validated prognostic system for ischemic stroke patients, comprising of a weighted score of 17 comorbidities based on the International Classification of Diseases, Ninth Revision, was determined.[38, 39] The degree of SAH on the first CT brain scan was evaluated according to the modified Fisher's grading and Hijdra scoring systems by another independent neurosurgeon.[40, 41] The Hijdra system consists of a semiquantitative assessment of the amount of blood identified in 14 regions of interest with a score of ≥ 22 being recently identified as an independent predictor for poor functional outcome, i.e. a modified Rankin score (mRS) of 4 to 6, at six-months.[42]

Functional outcome was evaluated by an independent nurse, without knowledge of the subject's group assignment, at 30 days, three months and six months after ictus using the following instruments: the Extended Glasgow Outcome Scale (GOSE), mRS (modified Rankin Scale) and the Barthel Index (BI). The primary endpoint was favorable GOSE performance, defined as grades 5 to 8 (moderate disability to good recovery), at six months after ictus. Multiple secondary study endpoints were also evaluated by an independent nurse for mRS, BI, neurocognitive function and quality of life at 30 days, three- and six-months. Good recovery was defined as a six-month mRS of 0 to 2 (asymptomatic to slight disability) and a six-month BI of 70 to 100 (mildly dependent to independent). Neurocognitive performance was evaluated by the Montreal Cognitive Assessment (MOCA) and the Neurobehavioural Cognitive State Examination (NCSE) also at the same time points. Quality of life (QoL) was appraised by adopting the Chinese version of the 36-item Short Form Health Survey questionnaire (SF-36) and the Stroke-specific QOL Scale (SS-QOL). The occurrence of cerebral vasospasm, DCI and radiological evidence of cerebral infarction (unrelated to the primary aneurysm treatment) were determined by an independent neurosurgeon and neuro-radiologist. Cerebral vasospasm was defined as angiographically detectable moderate-to-severe arterial narrowing not attributable to atherosclerosis, catheter-induced spasm or vessel hypoplasia. A TCD reading of the middle cerebral artery (MCA) with a mean velocity of > 120 cm/sec or a Lindegaard ratio (MCA: internal carotid artery mean velocity) of > 3 was also interpreted as indicative of cerebral vasospasm.[6] DCI was defined as a decrease in GCS of ≥ 2 points or the development of focal neurological deficit for at least one hour not related to post-treatment complications, rebleeding, hydrocephalus, infection, electrolyte or metabolic disturbances according to

Vergrouwen et al.[5] SAH-related cerebral infarction was defined as CT or MRI evidence of such within six weeks of ictus, that was absent on scans performed 24 to 48 hours after aneurysm occlusion and was not related to the neurosurgical procedure.[5] Finally, 30-day mortality, three- and six-month mortality were also recorded.

Safety Evaluation

Cerebrolysin-related severe adverse effects (SAEs) are rare. They are defined as hypersensitivity reactions such as anaphylactic shock, seizures and acute renal failure.[43] Other AEs are generally infrequent, transient and mild: agitation, headache, vertigo, gastrointestinal symptoms such dyspepsia, diarrhea, constipation, nausea and vomiting.[43] Patients assigned to receive Cerebrolysin were monitored by the treating clinician for any changes in vital signs as well as in their general physical and neurological examinations. Laboratory tests were also evaluated for abnormalities attributable to Cerebrolysin. If SAEs occurred the decision for premature trial termination was made by study investigators.

Statistical Analysis

Since the treatment effects of Cerebrolysin in aneurysmal SAH are unknown, to determine the sample size for this pilot study, trials focused on ischemic stroke were used as a reference. The largest phase III trial for Cerebrolysin in ischemic stroke determined that a sample size of 990 subjects, with an α -level of 0.025 (one-sided) and 90% power, was required to detect treatment superiority over standard care alone for functional performance at three months.[27] By adopting a Bayesian decision-theoretic approach, it was proposed that phase II trials should have a sample size approximately 0.03 times that of a subsequent phase III study, which in this case would be 30 ($= 990 \times 0.03$) subjects in total.[44] However, it was ultimately decided to increase the sample size to 50 subjects since Sim et al advocated that this number was the absolute minimum required to decide on whether to proceed with a main RCT with a 5% two-tailed α -level and 80% power.[45] All analyses were performed on a modified intention-to-treat basis where the last available observed outcome measure was carried forward to handle missing data. Prespecified subgroup analyses were performed for age (≥ 65 or < 65 years), pre-existing hypertension, modified WFNS grade (good grade: I, i.e. an admitting GCS of 15 or II, i.e. GCS of 14 versus poor grade: III, i.e. GCS 13; IV, i.e. GCS 7–12 or V, i.e. GCS 3–6), modified Fisher grading (I, II or III, IV), aneurysm location (anterior or posterior circulation) and treatment modality (endovascular therapy or clipping). The reporting of this study was in accordance with the recommendations outlined in the Consolidated Standards of Reporting Trials (CONSORT) statement.[46] Statistical tests included logistic regression, the chi-squared test, independent t-test and the Wilcoxon Mann-Whitney U-test. An α -level of 0.05 was used to define statistical significance. Tests were performed by either using Statistical Package for the Social Sciences software version 20.0 (SPSS Inc., Chicago, Illinois, USA) or R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 142 patients were diagnosed with aneurysmal SAH between 1 February 2014 and 26 June 2018. The most frequent reason for exclusion was unsalvageable neurological presentation (27, 19%) followed by the patient's age being older than 80 years (18, 13%) and delayed neurosurgical treatment beyond 96 hours after ictus (14, 10%) (Fig. 1). Among them 50 patients (35%) were enrolled into the pilot study with 25 assigned to the intervention or control groups respectively. All patients completed 14 days of Cerebrolysin administration, none withdrew from the study prematurely and there were no study protocol violations. Significant baseline characteristic differences between the two groups were not detected (Table 1). The mean time from ictus to Cerebrolysin or placebo infusion was 29.4 ± 14.8 hours. The mean age was 53 years ± 10 (range: 34–78 years) with a male: female ratio of 1:2. The median modified WFNS grade was II and the majority of patients presented with good-grade SAH (30, 60%) with a mean APACHE II score of 10 ± 5 . Regarding the amount of SAH on the initial CT scan, the median modified Fisher grade was III and the mean Hijdra score was 17 ± 7 . Most intracranial aneurysms were located at the internal carotid artery (ICA) (21, 42%) followed by the anterior communicating artery (ACoM) (12, 24%) and the middle cerebral artery (MCA) (10, 20%). The majority of patients underwent endovascular therapy (35, 70%) and the remaining were treated by clipping. No SAEs or mortality attributable to Cerebrolysin administration was observed. The drug did not interfere with standard-of-care management of aneurysmal SAH patients.

Table 1
Baseline Characteristics of Patients in the Cerebrolysin and Placebo Groups

	Cerebrolysin n = 25 (%)	Normal Saline n = 25 (%)	P-value
Patient Factors			
Age, mean \pm SD (range), years ≥ 65 years	52 \pm 9 (31–65) 2 (8)	54 \pm 11 (37–70) 4 (16)	0.72 0.67
Female	16 (64)	18 (72)	0.76
Smoker	12 (48)	9 (36)	0.39
Drinker	1 (4)	4 (16)	0.35
Pre-existing hypertension	11 (44)	5 (20)	0.07
Baseline MAP, mean \pm SD, mmHg	127 \pm 28	122 \pm 22	0.45
Median CCI	1	1	1.00
Disease Factors			
Modified WFNS	11 (44)	14 (56)	0.79
I	4 (16)	1 (4)	
II	1 (4)	3 (12)	
III	8 (32)	6 (24)	
IV	1 (4)	1 (4)	
V			
APACHE II score, mean \pm SD	11 \pm 5	9 \pm 5	0.14
Modified Fisher CT Grade	3 (12)	7 (28)	0.07
I	3 (12)	5 (20)	
II	12 (48)	6 (24)	
III	7 (28)	7 (28)	
IV			
Hijdra score, mean \pm SD	17 \pm 6	16 \pm 7	0.64
Hijdra score ≥ 22	6 (24)	8 (32)	0.53
Hydrocephalus	11 (44)	11 (44)	1.00
Intraventricular hemorrhage	10 (40)	10 (40)	1.00
Intracerebral hemorrhage	6 (16)	6 (16)	1.00
Rebleeding	0	1 (4)	1.00

N.B. MAP, mean arterial pressure; CCI, Charlson comorbidity index; WFNS, World Federation of Neurosurgical Societies; APACHE, Acute Physiology and Chronic Health Evaluation; IQR, interquartile range; CT, computed tomography; ICA, internal carotid artery; ACA, anterior cerebral artery; AComA, anterior communicating artery; MCA, middle cerebral artery; PC, posterior circulation

	Cerebrolysin n = 25 (%)	Normal Saline n = 25 (%)	P-value
Aneurysm location	10 (40)	11 (44)	0.53
ICA	5 (20)	9 (36)	
ACA/ AComA	7 (28)	3 (12)	
MCA	3 (12)	2 (8)	
PC			
Treatment Factors			
Aneurysm treatment	16 (64)	19 (76)	0.11
Endovascular therapy	9 (36)	6 (24)	0.36
Clipping			
N.B. MAP, mean arterial pressure; CCI, Charlson comorbidity index; WFNS, World Federation of Neurosurgical Societies; APACHE, Acute Physiology and Chronic Health Evaluation; IQR, interquartile range; CT, computed tomography; ICA, internal carotid artery; ACA, anterior cerebral artery; AComA, anterior communicating artery; MCA, middle cerebral artery; PC, posterior circulation			

Analyses were performed according to the original assigned study groups. A larger proportion of patients in the Cerebrolysin group had a favorable GOSE outcome at six months (76%, 19) compared to those that received saline (68%, 17), but the difference was not significant (OR 1.49; 95% CI 0.43–5.17) (Fig. 2 and Table 2). Similar findings were also observed for the six-month functional performance measures for both good mRS (0–2) and BI \geq 70 (Fig. 2 and Table 2).

Table 2
A Comparison of Outcomes in the Cerebrolysin and Placebo Groups

	Cerebrolysin n = 25 (%)	Normal Saline n = 25 (%)	OR (95% CI) or P-value (*)
Functional Outcome at 6 months	7 (5–8)	7 (3–8)	NS
GOSE, median (IQR)	19 (76)	17 (68)	0.53 (0.43–5.17)
GOSE, favorable outcome, i.e. 5–8	13 (52)	13 (52)	1.00 (0.33–3.03)
GOSE good outcome, i.e. 7–8	1 (0–3)	2 (0–4)	NS
mRS, median (IQR)	19 (76)	17 (68)	0.53 (0.43–5.17)
mRS good outcome, i.e. 0–2	91 ± 28	90 ± 24	0.90*
BI, mean ± SD	23 (92)	18 (72)	0.52 (0.29–12.72)
BI ≥ 70			
Mortality	0	3 (12)	0.88 (0.76–1.02)
30-day	0	4 (16)	0.46 (0.33–0.63)
3-month	0	4 (16)	0.46 (0.33–0.63)
6-month			
Delayed Cerebral Ischemia	11 (44)	12 (48)	0.85 (0.28–2.59)
Cerebral Vasospasm	3 (12)	7 (28)	0.35 (0.08–1.55)
Cerebral infarction	14 (56)	15 (60)	0.85 (0.28–2.61)
Neurocognitive Performance at 6 months	21 ± 9	21 ± 8	0.98*
MOCA, mean ± SD	15 (60)	10 (40)	1.65 (0.51–5.33)
MOCA ≥ 22	20 (80)	18 (72)	0.67 (0.14–3.19)
NCSE, within average range	21 (84)	18 (72)	0.88 (0.17–4.43)
Orientation	18 (72)	16 (64)	0.80 (0.21–3.04)
Attention	15 (60)	10 (40)	1.65 (0.51–5.33)
Language comprehension	11 (44)	3 (12)	4.71 (1.10–20.00)
Repetition	19 (76)	15 (60)	1.27 (0.34–4.74)
Naming	12 (48)	9 (36)	1.23 (0.38–4.00)
Constructional ability	19 (76)	17 (68)	0.75 (0.18–3.10)
Memory	18 (72)	10 (40)	2.83 (1.01–9.61)
Calculation	19 (76)	18 (72)	0.53 (0.11–2.43)
Reasoning			
Judgment			
Quality of Life at 6 months	77 ± 23	74 ± 26	0.60*
SF-36®	69 ± 25	69 ± 21	0.98*
Physical health score, mean ± SD	4.5 ± 0.7	4.5 ± 0.6	0.98*
Mental health score, mean ± SD	4.6 ± 0.8	4.5 ± 0.7	0.84*
SS-QoL, mean ± SD	4.2 ± 0.9	4.4 ± 0.6	0.64*
Physical subscore, mean ± SD			
Psychosocial subscore, mean ± SD			
Adverse Effects	0	0	NS
Length of Hospital Stay, mean ± SD	33 ± 15	39 ± 32	0.41*
OR, odds ratio; CI, confidence interval; mRS, modified Rankin score; GOSE, extended Glasgow outcome score; IQR, interquartile range; SD, standard deviation; BI, modified Barthel index; MOCA, Montreal cognitive assessment; NCSE, Neurobehavioral cognitive state examination; SF-36®, Short-form 36 Health Survey; SS-QoL, stroke-specific quality of life; NS, not significant			

30-day all-cause mortality was higher in the saline group (12%, 3) than in the Cerebrolysin group where all patients survived, but this was not significant (OR 0.88, 95% CI 0.76–1.02). However, a noticeably higher incidence in three- and six-month mortality was observed in saline group patients. 16% (4) of saline group patients died while none died by these time points in the Cerebrolysin group (both ORs 0.46; 95% CI 0.33–0.63). The cause of death for three of these patients (75%, 3/4) was due to complications arising from DCI and the remaining patient died from severe chest infection. A further review of the incidence of inpatient SAH-related complications such as cardiac failure, acute myocardial infarction, renal failure, chest infection, septic shock, pulmonary embolism or gastrointestinal bleeding revealed comparable frequencies of occurrence between the two study groups.

Overall, DCI developed in 46% (23) of patients and the proportion of patients in each group were similar (OR 0.85; 95% CI 0.28–2.59). Similar findings were also noted with regard to cerebral infarction (OR 0.85; 95% CI 0.28–2.61). Cerebral vasospasm was diagnosed in 20% (10) of all patients, with a higher proportion in the saline group (28%, 7) than in the Cerebrolysin group (12%, 3), but this did not reach statistical significance (OR 0.35; 95% CI 0.08–1.55).

Six-month neurocognitive and QoL assessments were feasible in 80% (40/50) of communicable patients, 21 (84%) patients in the Cerebrolysin group and 19 (76%) in the saline group. The mean MOCA scores were similar, 21 ± 9 in the Cerebrolysin group and 21 ± 8 in the saline group (95% CI -5.26-5.38). Most of the assessed domains for the NCSE were comparable, but patients in the Cerebrolysin group performed notably better in naming (OR 4.71; 95% CI 1.10–20.00) and in reasoning (OR 2.83; 95% CI 1.01–9.61). For six-month QoL assessments, mean SF-36 physical and mental scores as well as the mean physical and psychosocial subscores for SS-QoL were similar for both groups.

Predefined subgroup analyses for favorable six-month GOSE by age (cut-off at 65 years), pre-existing hypertension, WFNS grade, modified Fisher grading, aneurysm location and treatment modality also did not reveal significant superiority for Cerebrolysin (Fig. 3).

Discussion

The results from this study suggests that Cerebrolysin administration is safe, well tolerated and feasible for aneurysmal SAH patients. Cerebrolysin showed a satisfactory safety profile with no increased incidence of SAEs or mortality. There was also no interference with standard-of-care treatment according to AHA/ASA guidelines and administration within 96 hours of ictus, i.e. before the onset of DCI, was achievable.

One of the major reasons for the recurrent failure of several neuroprotective drug clinical trials to achieve positive outcomes in stroke was the investigation of a single agent's effect on a specific component in the ischemic cascade.[47, 48] In contrast, Cerebrolysin is the only brain-specific pleiotropic drug that targets multiple ischemic pathophysiological events, including counteracting glutamate excitotoxicity, neuroinflammation and apoptosis, due to the combined action of its several constituent neurotrophic factors.[18–25, 49–52] The exact mechanisms of action are unknown, but several preclinical studies

have suggested that Cerebrolysin not only possesses neuroprotective properties during the acute stroke period, but may also enhance neurorestoration during the post-stroke recovery phase.[24, 26, 28, 32, 53, 54]. For the latter, neuroplasticity manifested by synaptic remodeling and facilitated synaptic transmission have been observed.[52, 55] Murine ischemic stroke model studies have also detected Cerebrolysin-induced neural progenitor cell proliferation at the subventricular zone and neurogenesis with oligodendrogenesis in the peri-infarct zone by activation of the Sonic hedgehog and PI3K/Akt signaling pathways.[25, 49, 50] To our knowledge this is the first study in the literature to investigate the role of Cerebrolysin for aneurysmal SAH. In addition, no previous Cerebrolysin stroke study has reported the effect of the drug on six-month outcomes despite its asserted neurorestorative potential during the recovery phase.

Neuroprotective stroke trials have often been limited by inappropriately long time-windows for drug administration constituting an additional challenge in translational research.[47, 56] It was hypothesized that for aneurysmal SAH, the early administration of Cerebrolysin before the onset of DCI would have a prophylactic or ameliorating effect that would ultimately improve overall functional outcome. However, with regard to the primary endpoint for favorable six-month GOSE, there was no significant difference in the proportions of patients that received Cerebrolysin from those that had saline (p-value: 0.53). A priori subgroup analysis also did not identify patients that would more likely benefit from Cerebrolysin. A trend for lower 30-day mortality was observed for Cerebrolysin group patients (p-value: 0.07) and a significant survival benefit was observed for 3- and 6-month mortality (both p-values: 0.04). The cause of death for 75% (3/4) of these patients was due to DCI. But no significant reductions in the overall proportion of patients experiencing DCI (p-value: 0.78), cerebral vasospasm (p-value: 0.16) or infarction (p-value 0.77) were noted in Cerebrolysin group patients. The limitation in sample size could not allow for subgroup analysis to detect an association between DCI and mortality, but DCI has consistently been proven to be a major independent predictor for survival from several other studies.[5, 7–9, 37] One potential reason for this study's neutral results could be ascribed to underestimation of the impact early brain injury (EBI) which describes the pathophysiological cascade for neuronal cell death immediately following SAH. We believed that administering Cerebrolysin before the onset of DCI would have been sufficiently expeditious, but the concept of EBI has gained precedence as a therapeutic target in recent years.[57] SAH-induced EBI triggers blood-brain barrier dysfunction, inflammation, apoptosis, oxidative stress, cortical spreading depression and excitotoxicity, that can be at least equal or more predictive than DCI for mortality and morbidity.[57, 58] In comparison, investigators of neuroprotective drug trials for ischemic stroke have increasingly advocated the importance of ultra-early intervention within the first four to six hours of symptom onset and even in the prehospital setting.[47, 48, 56, 57] In support of earlier intervention for SAH neuroprotection, Cerebrolysin ischemic stroke RCTs have demonstrated significant improvements in three-month neurological outcomes when the drug was delivered within six hours after hospital admission and within one hour after completing systemic recombinant tissue plasminogen activator thrombolytic therapy.[30, 33] In the present study the mean duration for initiation of Cerebrolysin was 29 hours, well before the development of DCI which mostly occurs between days 4 and 10 of SAH, but we

propose that future studies should consider implementing an earlier intervention time-window to account for EBI.[5, 56]

Almost one-fifth of patients (19%, 27) were excluded from the study due to the initial clinical judgement that they were unlikely to survive for more than 48 hours or they exhibited signs of brain herniation. This may have led to an over-representation of patients with good WFNS grade SAH (60%, 30) and therefore a ceiling effect for favorable GOSE outcome regardless of whether Cerebrolysin was administered. Similar conclusions were reached by investigators of a large multicenter RCT for ischemic stroke that also produced neutral results for Cerebrolysin.[27] A recent meta-analysis also noted that the strongest therapeutic effects of Cerebrolysin were observed in trials with high baseline stroke severity (moderate to severe) as expressed by the National Institutes of Health Stroke Scale (NIHSS).[53] Since up to 26% of patients with poor WFNS grade V SAH can experience good long term mRS (0–2) outcomes we also recommend that subsequent studies consider recruiting patients with more severe SAH by assessing patient eligibility after neurological resuscitation.[59, 60]

The optimum dose for Cerebrolysin in the setting of aneurysmal SAH requires further investigation. Experimental studies investigating the effect of Cerebrolysin in murine models for focal ischemic stroke noted that a threshold daily dose of 2.5 ml/kg to 5 ml/kg was required before significant reductions in infarct volume or improvements in functional outcome could be observed.[23, 25] Apart from one clinical ischemic stroke trial that concluded a daily 50 ml dose of Cerebrolysin for 10 days resulted in better 30-day NIHSS scores than a lower dose of 10 ml, no other trial has compared different dosing regimens.[29] Even though we adopted a 30 ml daily dose protocol from previous positive ischemic stroke studies, since SAH induces more extensive cerebral ischemia and the minimally-effective weight-based drug dosages discovered from animal studies are considerably higher than that prescribed in human subjects, it is possible that Cerebrolysin group patients may have been undertreated which could have accounted for our neutral findings.

Cognitive dysfunction is gradually being recognized as an important source of disability in SAH survivors occurring in 7–15% of patients with DCI being a major predictor.[61, 62] Coupled with the positive clinical results of Cerebrolysin in treating vascular dementia and Alzheimer's disease, we also hypothesized that an improvement in neurocognitive performance could be detected in SAH patients, but no significant difference between the study groups existed.[63, 64] This may be due to the relatively short two-week course of administration that was designed to predominantly exploit Cerebrolysin's neuroprotective action. Conversely for dementia RCTs, four- to twelve-week Cerebrolysin regimens were selected to principally harness its neurorestorative capacity.[63, 65] Therefore we suggest that subsequent trials may need to incorporate a longer Cerebrolysin exposure duration, for example at least four weeks, to include the post-SAH recovery phase in order to comprehensively assess its potential benefits in neurocognition.

Conclusions

The findings from this trial support the safety and feasibility of administering Cerebrolysin to aneurysmal SAH patients. Although no significant benefit for functional performance could be detected, there is evidence to suggest that Cerebrolysin reduces three- and six-month mortality. Due to the exploratory nature of this study and its small sample size, these findings should be confirmed in an adequately powered clinical trial to detect group differences in other clinically meaningful endpoints such as DCI and neurocognitive performance. An earlier time-window for intervention, a longer duration of intervention to encompass the rehabilitation phase and the recruitment of a more homogenous group of moderate to severe WFNS grade (III to V) SAH patients may impart greater insight into Cerebrolysin's therapeutic role.

Abbreviations

AHA American Heart Association

APACHE Acute Physiology and Chronic Health Evaluation

ASA American Stroke Association

BI Barthel Index

CCI Charlson Comorbidity Index

CI Confidence interval

CONSORT Consolidated Standards of Reporting Trials

CT Computed tomography

DCI Delayed cerebral ischemia

EBI Early brain injury

GCS Glasgow Coma Scale

GOSE Glasgow Outcome Scale Extended

MCA Middle cerebral artery

mRS Modified Rankin Score

MOCA Montreal Cognitive Assessment

NCSE Neurobehavioural Cognitive State Examination

NIHSS National Institutes of Health Stroke Scale

OR Odds ratio

QoL Quality of Life

RCT Randomized controlled trial

SAE Severe adverse effect

SAH Subarachnoid hemorrhage

SF-36 36-Item Short Form Health Survey questionnaire

SS-QOL Stroke-specific QOL Scale

TCD Transcranial Doppler

WFNS World Federation of Neurosurgical Societies

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Clinical research ethics committee approval was obtained (Hong Kong Hospital Authority, Kowloon West Cluster Institutional Review Board number: KW/FR-13-006 (61-04)). All subjects or their legal representatives provided written informed consent. Patient data was anonymized during the subsequent analysis and dissemination.

CONSENT FOR PUBLICATION

All authors consented.

AVAILABILITY OF DATA AND MATERIALS

The datasets acquired and analyzed for this study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

PW: project execution, manuscript preparation, data acquisition, analysis, interpretation, review and critique. SL, JH, NK, RL and LJ: data acquisition and review. AC, MK, YC, AW, HTW, KYC and JK reviewed and critiqued the manuscript. All authors' have read and approved the manuscript.

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Figures



Figure 1

Trial patient profile



Figure 2

Distribution of 6-month GOSE and mRS functional outcomes



Figure 3

Forest plot. A priori subgroup analysis for favorable GOSE (5 to 8) at 6 months. "Events" indicates the number of patients with favorable 6-month GOSE for each subgroup. "Total" indicates the number of patients with a particular characteristic that were assigned to either the Cerebrolysin or Normal Saline (placebo) group. N.B. WFNS, World Federation of Neurosurgical Societies

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