Cerebrolysin and Recovery After Stroke (CARS)
A Randomized, Placebo-Controlled, Double-Blind, Multicenter Trial

Dafin F. Muresanu, MD, PhD; Wolf-Dieter Heiss, MD; Volker Hoemberg, MD;
Ovidiu Bajenaru, MD, PhD; Cristian Dinu Popescu, MD, PhD; Johannes C. Vester;
Volker W. Rahlfs, PhD; Edith Doppler, PhD, Dieter Meier, MD; Herbert Moessler, PhD;
Alla Guekht, MD, PhD, DMedSci

Background and Purpose—The aim of this trial was to investigate whether stroke patients who receive Cerebrolysin show improved motor function in the upper extremities at day 90 compared with patients who receive a placebo.

Methods—This study was a prospective, randomized, double-blind, placebo-controlled, multicenter, parallel-group study. Patients were treated with Cerebrolysin (30 mL/d) or a placebo (saline) once daily for 21 days, beginning at 24 to 72 hours after stroke onset. The patients also participated in a standardized rehabilitation program for 21 days that was initiated within 72 hours after stroke onset. The primary end point was the Action Research Arm Test score on day 90.

Results—The nonparametric effect size on the Action Research Arm Test score on day 90 indicated a large superiority of Cerebrolysin compared with the placebo (Mann–Whitney estimator, 0.71; 95% confidence interval, 0.63–0.79; $P<0.0001$). The multivariate effect size on global status, as assessed using 12 different outcome scales, indicated a small-to-medium superiority of Cerebrolysin (Mann–Whitney estimator, 0.62; 95% confidence interval, 0.58–0.65; $P<0.0001$). The rate of premature discontinuation was <5% (3.8%). Cerebrolysin was safe and well tolerated.

Conclusions—Cerebrolysin had a beneficial effect on function and global outcome in early rehabilitation patients after stroke. Its safety was comparable with that of the placebo, suggesting a favorable benefit/risk ratio. Because this study was exploratory and had a relatively small sample size, the results should be confirmed in a large-scale, randomized clinical trial.

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Key Words: Cerebrolysin ■ randomized, double-blind, placebo-controlled trial ■ recovery of function ■ rehabilitation ■ stroke

Ischemic stroke is the second most common cause of death worldwide and the third leading cause of the loss of disability-adjusted life years[1,2]; however, treatment remains insufficient and is only successful during the first hours after the attack if reperfusion of the ischemic territory can be achieved. Thrombolysis resulting from the intravenous administration of recombinant tissue-type plasminogen activator within 4.5 hours significantly reduces the incidence of death or dependency at 3 to 6 months, but the benefit of its administration ceases between 4.5 and 6 hours after the ictus.[3] Attempts to recanalize occluded vessels after this time window by intra-arterial recombinant tissue-type plasminogen activator or mechanical thrombectomy enhance reperfusion[4] and have recently been shown to improve clinical outcome in carefully selected patients.[5–7] However, the number of patients who may benefit from these reperfusion therapies is small and probably totals <20% of all stroke victims, even for those treated at specialized centers.[8,9]

Therefore, many therapeutic strategies have been developed targeting the pathophysiological cascade that starts with ischemia and ultimately leads to irreversible tissue damage. Despite beneficial results obtained in the development of

infarcts and in functional outcome following experimental ischemia,[10] neuroprotective drugs have not shown efficacy in clinical trials.[11–13] This failure to translate results from experimental studies to clinical application might be due in part to the use of inappropriate animal models[14] and also to the design of human trials, which often do not consider the limited time windows of targeted steps in the pathophysiological cascade or the complexity of the biochemical and molecular mechanisms leading to ischemic brain damage. As a consequence, treatments directed at correcting one biochemical or molecular step in the pathophysiological cascade of ischemic cell damage have not been successful in stroke, warranting the testing of a multitargeted therapy that includes compounds with effects on several of the associated pathophysiologic events. One of these multimodal compounds is Cerebrolysin, a neuropeptide preparation of porcine origin produced by a standardized manufacturing process and consisting of low molecular weight neuropeptides (<10 kDa) and free amino acids. Cerebrolysin has been shown to have neuroprotective properties and to be effective against excitotoxicity, inhibiting free radical formation, microglial activation/neuroinflammation, and calpain activation/apoptosis, and additionally, it has been demonstrated to exhibit neurotrophic activity, promote neuronal sprouting, improve cellular survival, and stimulate neurogenesis.[15–19] This therapeutic approach has shown success in experimental middle cerebral artery occlusion models, resulting in a reduction in the infarction volume and improvement of functional recovery.[20–23] In animal models, an improved neurological outcome has been observed, even when Cerebrolysin administration has been started in the subacute stages of the stroke, that is, ≤48 hours after the onset of symptoms.[21,23] Thus, the neuroplastic, recovery-promoting effects of this compound prompted a much broader window of opportunity for clinical studies, as has been suggested for neuroprotective treatment. Cerebrolysin has been tested in several clinical trials during the acute phase after ischemic stroke,[24–27] but these studies have had small sample sizes mainly ranging from 50 to 200 randomized patients. Based on the data of a larger, randomized, double-blind, placebo-controlled trial, a post hoc subgroup analysis (n=252) has indicated a trend in favor of Cerebrolysin for improved outcome in patients with more severe stroke (National Institutes of Health Stroke Scale [NIHSS]>12) and a reduction in mortality.[28] The treatments in these previous clinical trials were initiated during the acute phase after stroke and were mainly limited to 10 days. The neuroprotective effects of Cerebrolysin have been primarily assessed, and its neurotrophic and neuroplastic effects on recovery, as indicated in animal experiments, have been neglected.[21,23] The efficacy of a longer duration of drug application has not been investigated.[29,30] The purpose of this Cerebrolysin and Recovery After Stroke (CARS) trial was to analyze the efficacy and safety of Cerebrolysin during recovery after stroke.

Methods

Study Design and Treatment Regimen

This prospective, randomized, double-blind, placebo-controlled, multicenter, parallel-group study compared the effects of 30 mL Cerebrolysin versus placebo during early rehabilitation after stroke.

Cerebrolysin was diluted with physiological saline to a total volume of 100 mL, and physiological saline (100 mL) was given as a placebo. The study medication was administered once daily for 21 days as an intravenous infusion for 20 minutes, beginning at 24 to 72 hours after stroke onset. In previous studies, drug dosages from 10 to 50 mL per day were used, and the treatment periods ranged from 10 to 30 days, with once-daily infusions of Cerebrolysin.[15–28,31]

Each patient included in our study participated in an accompanying standardized rehabilitation program for 21 days, beginning within 48 to 72 hours after stroke onset (5 d/wk for 2h/d). This program included massages and passive and active movements of the upper and lower limbs. The patients continued with 2×15 minutes of active movement for 3 days per week after discharge. The primary study end point was day 90. Study visits were conducted at 7, 14, and 21 days after baseline and on days 42 and 90 post stroke. The study duration for each patient was 90 days. The study was performed in Romania, Ukraine, and Poland, and it is registered with EudraCT (2007-000870-21).

The relevant institutional ethics committees approved the study, and all subjects provided informed consent. Patients with dysphasia limiting understanding of the informed consent were not included in the trial. All study procedures were conducted in accordance with the applicable laws and guidelines, Good Clinical Practice, and ethical standards.

Inclusion and Exclusion Criteria

Patients between 18 and 80 years of age were included in this trial. Only ischemic supratentorial strokes (confirmed using computed tomography or magnetic resonance imaging) with a volume of >4 cm³ were included. The patients included in the study had no significant prestroke disability (prestroke modified Rankin Scale [mRS] score, 0–1), had not experienced a stroke within the previous 3 months, and had an Action Research Arm Test[32] (ARAT) score of <50 (score ranging from 0 [no function] to 57 [no functional limitation]) and a Goodglass and Kaplan Communication Scale[33,34] score of >2 (score ranging from 0 [severe aphasia] to 5 [minimal aphasia]).

Patients were excluded for the following reasons: progressive or unstable stroke; a preexisting or active major neurological or psychiatric disease; a history of significant alcohol or drug abuse within the previous 3 years; advanced liver, kidney, cardiac, or pulmonary disease; a terminal medical diagnosis with an expected survival of <1 year; a substantial decrease in alertness at the time of randomization; any condition that would represent a contraindication for Cerebrolysin administration, including allergy; pregnancy or lactation; or participation in another therapeutic study of stroke or stroke recovery.

Randomization and Blinding

Treatments were assigned according to a predefined randomization plan. A study-specific randomization code was prepared using SAS software package (proc plan) in a validated working environment. A block size of 4 was used, and treatment assignments within each block were stratified by the clinical center at a ratio of 1:1. The block size was not known to the centers. Each center received medication for a sequence of complete blocks, and treatments were balanced within each center. Patients, healthcare providers, data collectors, outcome assessors, and the sponsor were blinded to the treatment allocation. The statistician in charge of randomization was unblinded, as was the person in charge of preparing the study medication, who received center-specific randomization envelopes and was independent of all other study-specific procedures, particularly any safety or efficacy assessments. Because Cerebrolysin has a slightly yellow tint, infusion bags were provided in sealed colored plastic sleeves to maintain the blinding.

Efficacy Criteria

The primary efficacy criterion was a change in the ARAT[32] score, and it was used to assess upper limb motor function from baseline to day

90. The secondary efficacy criteria were changes from baseline to day 21 (the last day on which the study medication was administered) and to day 90 in gait velocity (gait velocity test), fine motor function (9-Hole Peg test), the global neurological state (NIHSS), the level of disability or dependence in activities of daily living (Barthel Index, mRS), the extent of aphasia (Goodglass and Kaplan Communication Scale),[33,34] the extent of neglect (line cancellation test, gap detection test), quality of life (Short Form 36 items [SF-36] Health Survey, physical component summary, and mental component summary), and the extent of depression (Geriatric Depression Scale). References for these criteria are available in the online-only Data Supplement.

Statistical Methods

The primary objective of this trial was to investigate the hypothesis that patients randomized to Cerebrolysin would show improved ARAT scores over the 90 days of the study compared with those randomized to the placebo. The multiple level α of the study (the global level of significance for the entire study) was set to $\alpha=0.05$ (2-sided test for superiority). As planned before the study, nonparametric analyses were performed using the Wilcoxon–Mann–Whitney test because of the skewness and non-normality of the distributions (Shapiro–Wilk; $P=0.0137$) and the presence of outliers.

The Mann–Whitney estimator (MW) was calculated as the effect size measure associated with the well-known Wilcoxon–Mann–Whitney test.[35–38] Technically, the MW represents the probability that a randomly chosen subject from the test group is better off than a randomly chosen subject from the comparison group (with probability ranging from 0 to 1, and 0.5 indicating equality), and it is statistically defined as follows: $P(X<Y)+0.5\ P(X=Y)$.

The null and alternative hypotheses for the comparison of the effects of Cerebrolysin versus placebo can be formulated as follows (superiority test; T: test treatment; C: control treatment):

Null hypothesis H_0: MWTC$\leq$0.50.

Alternative hypothesis H_A: MWTC$>$0.50.

The traditional benchmark values[39,40] for the MW are 0.29 (large inferiority), 0.36 (medium inferiority), 0.44 (small inferiority), 0.50 (equality), 0.56 (small superiority), 0.64 (medium superiority), and 0.71 (large superiority).

In addition to univariate analysis of the ARAT score, a multidimensional approach to outcome assessment and classification was used to analyze the combined primary and secondary efficacy criteria because it is possible that no single measure can capture the multidimensional nature of recovery from stroke. The use of multiple measures to address the breadth of potential deficits and recovery after stroke has also been recommended by leading researchers.[41–43]

Multidimensional analyses were performed using the Wei–Lachin procedure, as described by Wei and Lachin[44] and Lachin.[45] This procedure is a multivariate generalization of the Wilcoxon–Mann–Whitney test that takes into account the correlations among univariate Mann–Whitney tests for each outcome to produce an overall, average estimate of benefit and test for treatment differences. The summarizing test used in this procedure is a directional test that is most efficient in cases in which the direction of superiority is known. However, the use of this test in a complex, heterogeneous disorder, such as stroke, has not yet been validated and needs further experience.[46]

Because of the exploratory nature of this phase II study, a formal sample size calculation, similar to that performed for confirmatory trials, was not conducted. However, an informal sample size calculation for the envisaged enrollment of 2×112 subjects resulted in 80% power (type II error rate of $\beta=0.20$) to detect a standardized mean difference of 0.376 with a significance level (type I error rate) of $\alpha=0.05$ (2-sided t test; nQuery Advisor, release 6.0).

All analyses were performed on a modified intention-to-treat (mITT) analysis set using the last observation carried forward (LOCF) approach for handling missing data. The mITT analysis set was defined as all randomized patients who have had at least 1 dose of study medication and have assessments for the primary end point at baseline and at least 1 time point after the first dose of study medication.

Sensitivity analyses were performed using the observed cases (OC) approach. No patient in the mITT population had a major protocol violation; thus, the mITT population and the per-protocol population were identical. Before the study, primary subgroup analysis was defined for patients with ARAT baseline scores of >0 (results are available in the online-only Data Supplement).

Results

Study Population

A total of 208 patients were enrolled in this study between April 2008 and September 2010. All patients received at least 1 dose of the study medication or a placebo (Cerebrolysin, n=104; placebo, n=104), and thus, they represent the safety analysis set. A total of 12 patients discontinued participation in the study prematurely because of adverse events (AEs; Cerebrolysin, n=2; placebo, n=5), withdrawal of their consent (Cerebrolysin, n=2; placebo, n=2) or for administrative reasons (placebo, n=1). Three of these patients, who were all in the placebo group, had no postbaseline data and were thus excluded from the mITT analysis set. There were no other major protocol violations in the mITT population; thus, the mITT and per-protocol analysis sets both consisted of 205 patients (Cerebrolysin, n=104; placebo, n=101). Efficacy data for 5 patients were missing for day 90. Thus, the OC population was composed of 200 patients (96.2% of randomized patients and 97.6% of mITT patients), which is above the recommended benchmark of 90% for class I evidence-based quality studies.[47–49] There were no relevant group differences observed at baseline (Tables 1 and 2). The mean age of the patients was 64 years, 63.9% of the patients were men, and the mean NIHSS score was 9.2 (median of 8.0).

Primary Efficacy Criterion (ARAT Score)

The ARAT scores increased from 10.1±15.9 (0.0, 21.5) at baseline (arithmetic mean±SD; median, IQR) to 40.7±20.2 (51.0, 28.0) on day 90 in the Cerebrolysin group and from 10.7±16.5 (2.0, 18.0) to 26.5±21.0 (27.0, 44.0) in the placebo group (Figure 1A). The mean absolute changes in the ARAT scores at 90 days post stroke compared with those at baseline were 30.7±19.9 (32.0, 36.5) for Cerebrolysin and 15.9±16.8 (11.0, 22.0) for the placebo. An increase in the ARAT score was observed in 96 of 104 (92.3%) of the Cerebrolysin-treated patients versus 85 of 101 (84.2%) of the placebo-treated patients.

The time course of the OC approach was similar to the results of LOCF analysis, with final median ARAT score of 51.0 in the Cerebrolysin group and 22.0 in the placebo group (Figure I in the online-only Data Supplement). The handling of missing data had a negligible impact on the results because of the low dropout rates in both groups.

A nonparametric evaluation was performed as planned before the study was conducted because the data were expected to violate common parametric analysis assumptions, such as a normal distribution. Nonparametric LOCF analysis demonstrated a large superiority of Cerebrolysin relative to the placebo on day 90, with an MW=0.71 (95% confidence interval, 0.63–0.79; Figure 1B). The OC analysis results were in support of the LOCF results, with an MW=0.71 (95% confidence interval, 0.63–0.79). The time course revealed a

Table 1.   Demographic Baseline Characteristics (Safety Analysis Set)

Parameter	Total, n=208	Cerebrolysin, n=104	Placebo, n=104
Male sex, n (%)	133 (63.9)	70 (67.3)	63 (60.6)
Right-handed, n (%)	199 (95.7)	99 (95.2)	100 (96.2)
Mean age, y (SD)	64.0 (10.2)	64.9 (9.8)	63.0 (10.6)
Mean BMI, kg/m^2 (SD)	27.4 (4.2)	27.2 (4.1)	27.6 (4.3)
Mean time until treatment initiation, h (SD)*	53.2 (12.3)	51.9 (12.7)	54.6 (11.7)
Thrombolytic treatment, n (%)	4 (1.9)	2 (1.9)	2 (1.9)
Prevalence of risk factors, n (%)			
Hypertension	173 (83.2)	86 (82.7)	87 (83.7)
Hyperlipidemia	105 (50.5)	55 (52.9)	50 (48.1)
Diabetes mellitus	39 (18.8)	19 (18.3)	20 (19.2)
Arrhythmia	54 (26.0)	26 (25.0)	28 (26.9)
Coronary artery disease	83 (39.9)	38 (36.5)	45 (43.3)
Past/current smoker	67 (32.2)	33 (31.8)	34 (32.7)

BMI indicates body mass index.
*Calculated from stroke onset.

constant increase in the effect size, which peaked on day 90 (data not shown).

Sensitivity analyses for ARAT values of >0 at baseline and values of 3 to 54 at baseline were performed as well as stratified analyses for age, sex, and baseline ARAT score. The results of these sensitivity analyses were consistent with those of primary analysis and all stratified analyses supported the result of the unadjusted analyses (Figures II–VI in the online-only Data Supplement).

Secondary Efficacy Criteria and Global Outcome

Similar to the results of univariate analyses of ARAT scores (Figure 1B), substantial differences were found between the Cerebrolysin and placebo groups.

A favorable mRS score of 0 to 1 was found in 42.3% of the patients in the Cerebrolysin group compared with 14.9% of those in the placebo group, and similar results were found for mRS scores of 0 to 2 (the full distribution of mRS scores is provided in Figure 2).

A medium superiority (MW≥0.64) of Cerebrolysin was observed for 6 of the 12 efficacy criteria, including the ARAT, NIHSS, Barthel Index, mRS, short form 36 items physical component summary, and depression (Geriatric Depression Scale) scores (Figure 3). Small superiority of Cerebrolysin was demonstrated using the gait velocity test, 9-Hole Peg test, Goodglass and Kaplan Communication Scale, and the short form 36 items mental component summary (MW≥0.56). The proportions of patients, who exhibited neglect at baseline, were low in both groups (Cerebrolysin, n=9; placebo, n=10); an effect of Cerebrolysin on neglect was not observed (the line cancellation test and gap detection test).

The combined results (the global outcome using the Wei–Lachin procedure) revealed a small superiority of Cerebrolysin compared with the placebo, with an MW effect size of 0.62 (95% confidence interval, 0.58–0.65). The OC analysis results supported the LOCF results, with an MW=0.61 (95% confidence interval, 0.58–0.65; data not shown).

Safety and Tolerability

A total of 93.8% of the treated patients received 21 infusions (Cerebrolysin, 96.2%; placebo, 91.3%). Of the patients treated with Cerebrolysin, 69.2% reported at least 1 AE compared with 71.2% of the patients in the placebo group. Most of the AEs were rated as mild in severity (Cerebrolysin, 76.1%; placebo, 69.8%). An overview of the most frequent treatment-emergent adverse events reported in at least 5% of the patients in any group is shown in Table 3. Three patients in the Cerebrolysin group (2.9%) and 7 in the placebo group (6.7%) had serious adverse events (SAEs), none of which appeared related to the study medications (Table 4). The SAEs in the Cerebrolysin group were described as severe peripheral ischemia, moderate renal colic, and acute myocardial infarction, and all these SAEs resolved during the study period. Four patients (3.8%) in the placebo group died because of sepsis with acute renal failure and coma, sepsis with multiorgan failure, intestinal ischemia, and subdural plus intracerebral hematoma. No patient died in

Table 2.   Baseline Values of Efficacy Criteria (mITT)

Efficacy Criterion	Cerebrolysin, n=104	Placebo, n=101
ARAT (paretic side)		
Mean±SD	10.1±15.9	10.7±16.5
Median (IQR)	0.0 (21.5)	2.0 (18.0)
NIHSS		
Mean±SD	9.1±3.2	9.2±3.2
Median (IQR)	8.0 (4.0)	8.0 (5.0)
Barthel Index		
Mean±SD	35.5±24.9	35.4±24.6
Median (IQR)	30.0 (40.0)	30.0 (40.0)
Modified Rankin Scale score		
Mean±SD	3.9±0.8	3.9±0.8
Median (IQR)	4.0 (0.0)	4.0 (1.0)

ARAT indicates Action Research Arm Test; IQR, interquartile range; mITT, modified intention-to-treat; and NIHSS, National Institutes of Health Stroke Scale.
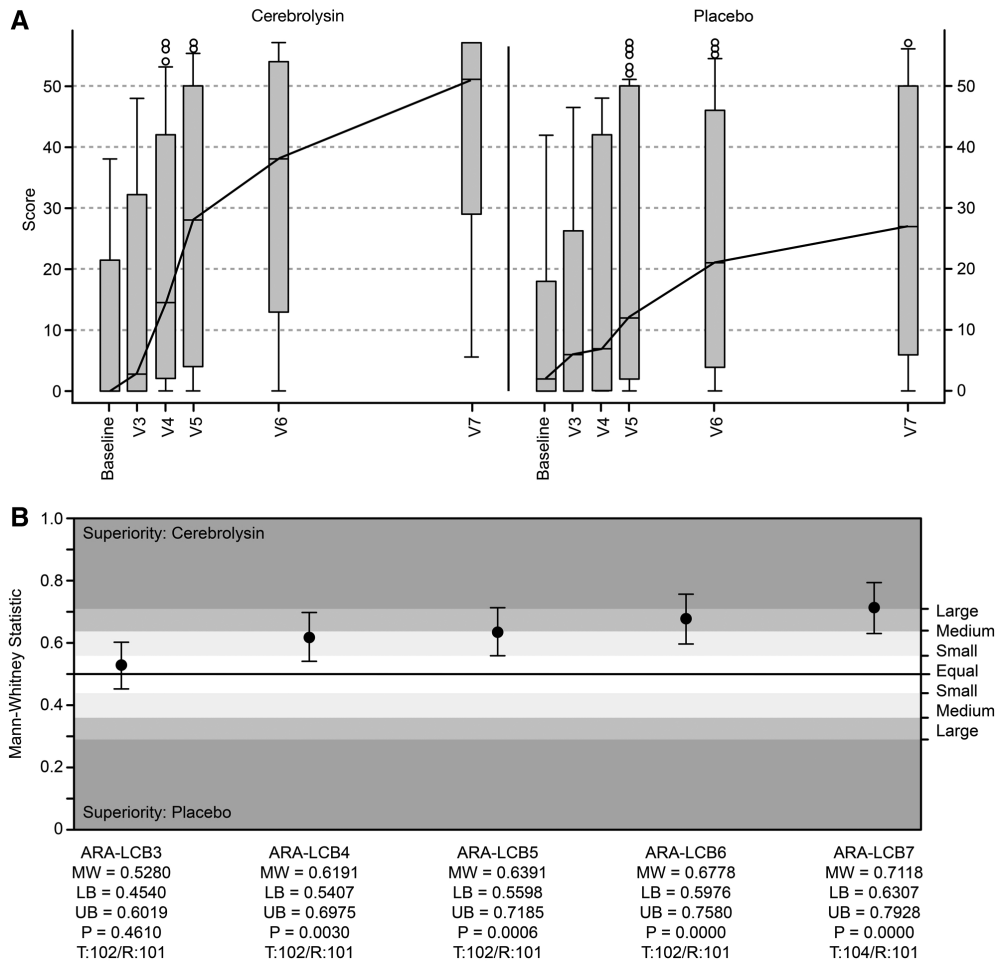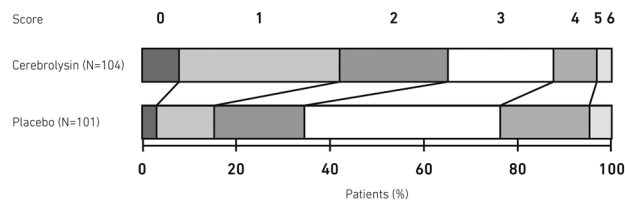
Figure 1. A, Time course of the Action Research Arm Test (ARAT) with Cerebrolysin (30 mL/d) and the placebo, shown as boxplot diagrams (P10 and P90) for days 7 (V3), 14 (V4), and 21 (V5) post baseline and days 42 (V6) and 90 (V7) post stroke. The modified intention-to-treat (mITT) population was analyzed using the last observation carried forward (LOCF) approach for handling missing data. The mITT-LOCF population on day 90 included a total of 205 patients (Cerebrolysin, n=104; placebo, n=101). **B**, Effect sizes (Mann–Whitney) of the ARAT score changes from baseline in the mITT-LOCF population. Analyses were conducted using the Wilcoxon–Mann–Whitney test.

the Cerebrolysin group. The low rate of SAEs can possibly be explained by the long duration of hospitalization (22–23 days for each patient according to the protocol). In addition, previous clinical studies have shown that early rehabilitation can prevent

Figure 2. Distribution of modified Rankin Scale scores. Cumulative percentage (Cerebrolysin vs placebo): 8.65 vs 2.97 (0), 42.31 vs 14.85 (1), 65.38 vs 33.66 (2), 88.46 vs 75.25 (3), 98.08 vs 96.04 (4), and 100.0 vs 100.0 (5). Definitions of scores: 0=no symptoms at all; 1=no significant disability despite symptoms: able to carry out all usual duties and activities; 2=slight disability: unable to carry out all previous activities but able to look after own affairs without assistance; 3=moderate disability: requiring some help, but able to walk without assistance; 4=moderately severe disability: unable to walk without assistance and unable to attend to own bodily needs without assistance; 5=severe disability: bedridden, incontinent, and requiring constant nursing care and attention; and 6=dead.

acute stroke complications, such as deep venous thrombosis, bronchopneumonia, pressure ulcers, and depression, which are the main sources of SAEs during the acute phase of stroke.[50–56]

The vital signs were similar between the treatment groups, and these factors did not show clinically relevant changes during the course of the study. The laboratory values classified by the investigators as clinically relevant did not exhibit any significant differences between the treatment groups, and no trends toward specific pathological laboratory findings were detected. Overall, the safety outcome reflected the expected safety and tolerability of patients after acute ischemic stroke.

Discussion

The results of this randomized, placebo-controlled, multicenter trial of stroke patients during early rehabilitation demonstrate beneficial effects of Cerebrolysin compared with a placebo on the primary efficacy criterion, the ARAT score, and on global outcome after 90 days. The ARAT score and global outcome were significantly different as determined by the preplanned first-line analysis and preplanned primary subgroup analysis of patients with ARAT baseline scores of >0. These findings were consistently observed in LOCF and OC
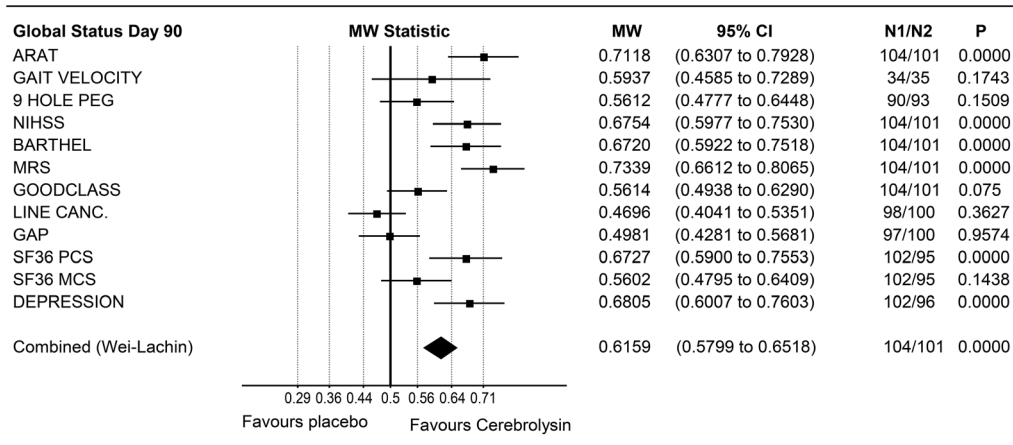
Figure 3. Global status on day 90. The effect sizes (Mann–Whitney [MW]) for the single and combined (Wei–Lachin procedure) efficacy parameters reflect changes from baseline in the modified intention-to-treat–last observation carried forward population (n=205). Analyses were conducted using the multivariate, directional Wilcoxon test. MCS indicates mental component summary; mRS, Modified Rankin Scale; and PCS, physical component summary.

sensitivity analyses. Negligible differences in the benchmark for equality were detected for premature discontinuation in the patients with AEs, those with at least 1 treatment-emergent adverse event and those with at least 1 SAE.

This study primarily recruited patients with moderate-to-severe stroke (median initial NIHSS score of 9) because a hypothesis-generating subgroup analysis of a previous study[28] indicated a trend for better outcome after Cerebrolysin treatment in patients with NIHSS >12 (n=246). This subgroup analysis has revealed that Cerebrolysin-treated patients show an improvement of 3 points higher on the NIHSS on day 90 compared with placebo-treated patients and has reported effect sizes demonstrating a medium superiority of Cerebrolysin relative to placebo for all domains of the composite end point (NIHSS, Barthel Index, and mRS). In the present trial, the Cerebrolysin group showed marked and significant improvements compared with the placebo group, and these patients achieved the highest ARAT scores.

Notably, the current trial also confirms the findings of a previous study in which Cerebrolysin was administered for 10 days as an add-on therapy together with intravenous recombinant tissue-type plasminogen activator treatment, resulting in a marked initial improvement.[27] However, the differences between these 2 groups vanished over time in the previous study and were not significant at 90 days after stroke with 30.4% of patients in the Cerebrolysin group having no symptoms at all (mRS score of 0) compared with 23.7% of those in the placebo group. No significant disabilities were observed in 21.4% of the Cerebrolysin-treated patients and in 28.8% of the placebo-treated patients, despite the presence of symptoms (mRS score of 1). The beneficial effects of Cerebrolysin were stable over the longer treatment period of 21 days in the present trial. We did note a poor rate of full recovery of the placebo patients in this trial. Generally, a poorer outcome than typically expected of the control group can explain the superiority of the treatment arm. However, this study primarily recruited patients with moderate-to-severe stroke (median initial NIHSS score of 9) and this could explain the low rate of spontaneous recovery under placebo. However, this possibility will need to be confirmed in a larger randomized trial. The results of this CARS trial cannot be directly compared with those of previous Cerebrolysin studies because both groups were actively exposed to rehabilitation intervention in this study. In addition, the initiation of rehabilitative therapy earlier may have played a role in the observed outcomes, as indicated by the more rapid initial clinical improvement. The neurorestorative activity of Cerebrolysin may also enhance the beneficial effects of rehabilitation.

This study was planned as an exploratory phase II trial. This design limits the degree of evidence obtained; thus, the results should be confirmed in a large-scale phase III trial. In addition, the generalizability of our results to other regions and stroke populations should be evaluated in future research.

The validity, sensitivity, and interrater and intrarater reliability of the primary efficacy criterion ARAT have been reported to be high.[32,57,58] However, each of these values represents reliability as assessed within a single institution. Increasingly, multisite trials of acute stroke have highlighted the importance of reducing the intersite variance that is present when assigning scores for outcome assessments.[59]

Table 3.   Most Frequently Reported TEAEs (in ≥5% of Patients; Safety Analysis Set)

Preferred Term	Cerebrolysin, n=104 n (%) freq	Placebo, n=104 n (%) freq
Urinary tract infection	13 (12.5) 15	17 (16.3) 18
Depression	11 (10.6) 11	10 (9.6) 10
Insomnia	6 (5.8) 6	4 (3.8) 4
Carotid arteriosclerosis	5 (4.8) 5	5 (4.8) 5
Headache	6 (5.8) 8	3 (2.9) 3
Carotid artery stenosis	6 (5.8) 6	2 (1.9) 3
Hypertension	9 (8.7) 15	12 (11.5) 18
Cytolytic hepatitis	10 (9.6) 10	8 (7.7) 8
Upper abdominal pain	6 (5.8) 6	4 (3.8) 5

Patients were counted only once for a particular AE. The TEAEs were coded according to MedDRA 13.1. Freq indicates the frequency with which each event was reported; and TEAEs, treatment-emergent adverse events (newly occurred or worsened under study treatment).

Table 4. Safety Outcome (Safety Analysis Set)

Safety Parameter	Total, n=208	Cerebrolysin, n=104	Placebo, n=104
Mean duration of exposure, d	20.4	20.5	20.3
Patients with TEAEs, n (%)	146 (70.2)	72 (69.2)	74 (71.2)
Drug-related, n (%)	44 (21.2)	22 (21.2)	22 (21.2)
Leading to drug withdrawal, n (%)	7 (3.4)	2 (1.9)	5 (4.8)
Number of TEAEs, n	400	201	199
Patients with TESAEs, n (%)	10 (4.8)	3 (2.9)	7 (6.7)
Drug-related, n (%)	0	0	0
Leading to drug withdrawal, n (%)	6 (2.9)	1 (1.0)	5 (4.8)
Number of TESAEs, n	16	3	13
Patients who died, n (%)	4 (1.9)	0	4 (3.8)

TEAEs indicates treatment-emergent adverse events (newly occurred or worsened under study treatment); and TESAEs, treatment-emergent serious adverse events.

The results of sensitivity analysis of the ARAT score are in line with those of primary analysis, indicating that the variations observed in the patients with ARAT scores of 0 at baseline had no relevant impact on the study outcome.

Considering that patients with lacunar or subtentorial stroke were excluded from this study, an analysis of stroke subtypes according to the affected vascular territory was not performed.

Conclusions

This study provides evidence that Cerebrolysin has beneficial effects on function and global outcome in early rehabilitation patients after stroke. All preplanned analyses generated statistically significant results. The high frequency of patients with ARAT baseline scores of 0 may limit the generalizability of the mITT results; however, preplanned subgroup analysis of the patients with ARAT baseline scores of >0 showed comparable effect sizes, supporting the positive overall results. The safety of Cerebrolysin was comparable with that of the placebo, suggesting that Cerebrolysin possesses a favorable benefit/risk ratio.

However, the design of the study limits the degree of evidence obtained. Caveats might result from limitations of any phase II study: small sample size, heterogeneity of populations, lack of central review of key end points, and possible imbalance in treatment groups not identifiable through routine risk factor descriptions. Thus, the results should be confirmed in a large-scale phase III trial. In addition, the generalizability of our results to other regions and stroke populations should be evaluated in future research.

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