THE INFLUENCE OF NEUROTROPHIC FACTORS TREATMENT ON STROKE VOLUME

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ABSTRACT

Background. Cerebrolysin is a multimodal drug, with pleiotropic neuroprotective effects that simultaneously promotes neuroprotection while retaining the ability to influence neuroplasticity. Cerebrolysin is approved for the treatment of stroke, traumatic brain injury and cognitive dysfunction.
Methods. A randomized, double-blind, placebo-controlled clinical trial was conducted in sixty patients with acute supratentorial ischemic stroke. The patients were randomized to either Cerebrolysin 30 ml/day (n = 30) or placebo (n = 30) in a 1:1 manner. The treatment period was 10 days. Stroke volume was measured in the first 24 hours after stroke onset using diffusion-weighted images, and it was measured again at 30 days after stroke using fluid-attenuated inversion recovery images.
Results. A significant reduction of stroke volume at 30 days was demonstrated in both Cerebrolysin- and placebo-treated patients ($p < 0.001$). Although it was not statistically significant a better response in stroke volume reduction was observed in the Cerebrolysin-treated group, compared to the placebo group (Mann Whitney test, $U = 426$, $p = 0.723$, mean rank, 29.70 vs. 31,30).
Interpretation. We demonstrated a positive effect from Cerebrolysin treatment to reduce ischemic stroke volume.

Key words: MRI stroke volume, Cerebrolysin, neuroprotection

INTRODUCTION

Stroke continues to represent one of the most important causes of mortality and persistent disability across the globe, despite impressive therapeutic progress within the field (1,2).

The only approved therapy for acute ischemic stroke is a recombinant tissue plasminogen activator (rt-PA) (3). Intravenous administration of rt-PA targets recanalization to reduce the size of ischemic damage, thereby improving the functional outcome. The major limit of a thrombolytic approach is the narrow therapeutic window (3). Despite the well-known positive effects of thrombolysis, only 2-5% of patients with acute ischemic stroke are treated with a thrombolytic approach, largely due to the limited therapeutic time window of 4,5 hours (3,4,5). Thus, it is critical to develop new strategies to improve the functional outcome after a stroke.

Neuroprotection and neurorecovery represent some of the most extensively studied therapeutic approaches in stroke treatment (6). The objective of neuroprotection is to protect neurons from the penumbra. This protection can be achieved by interfering with the ischemic cascade by modulating but not suppressing complex pathophysiological processes (6). More than one thousand compounds

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with potential neuroprotective effects have been evaluated in the last 20 years (7). These compounds have all failed to demonstrate efficacy in phase III clinical studies (3,7,8), but a positive trend was demonstrated in phase II clinical trials for some compounds, such as Cerebrolysin (9,10), citicoline (8,11), albumin (12) and granulocyte colony-stimulating factor (13).

There are many potential explanations for the failure to translate promising results from experimental trials to phase III clinical studies. Some differences in outcome may be related to stroke models or methodology, whereas other differences may be related to selection of patients, route and dosage, therapeutic time window, study design and analysis (6,7,14,15).

Classic predictors of functional outcome after stroke include the age of the patient (16), initial neurological deficit measured using the National Institute of Health Stroke Scale (NIHSS) (16,17) and the initial stroke size on diffusion-weighted imaging (DWI) (18). The question addressed in our study was whether these factors were able to predict and measure the neuroprotective and neurorecovery effects of Cerebrolysin in acute ischemic stroke patients.

Cerebrolysin is a peptide preparation produced by a biotechnological process, including a standardized enzymatic breakdown of purified, lipid-free brain proteins. The drug consists of low molecular weight neuropeptides (<10 kDa) and free amino acids (10).

Cerebrolysin is a multimodal, pleiotropic drug. A multimodal drug is able to simultaneously promote neuroprotection while retaining the ability to influence neuroplasticity (6). Due to its pleiotropic effects, Cerebrolysin modulates but does not suppress pathological processes, such as excitotoxicity, inflammation and apoptotic-like processes (6,10).

Cerebrolysin mimics the actions of neurotrophic factors and exerts neuroprotective and neurotrophic effects (10,19,20). Evidence for the anti-excitotoxic effects of Cerebrolysin are supported by its interactions with receptors of inhibitory neurotransmitters; the anti-oxidant effects are supported by its ability to block the formation of free oxygen radicals and to interfere with the gene expression of antioxidant enzymes (10). Cerebrolysin supports the survival of neurons, stimulates neuronal differentiation, growth and sprouting, supports the formation of synaptic contacts and enhances neurogenesis in the dentate gyrus of the hippocampus (21,22).

METHODS AND MATERIALS

Patients

Sixty patients (n = 60) with acute supratentorial ischemic stroke were enrolled in a double-blind, placebo-controlled, randomized clinical trial. The study was conducted according to ICH-GCP guidelines and approved by the local ethics committee.

The patients were randomized to either Cerebrolysin 30 ml/day (n = 30) or placebo (n = 30) in a 1:1 manner.

The diagnosis of the supratentorial ischemic stroke as well as the location of the stroke were determined clinically and was confirmed by imaging (Computed Tomography (CT) or Magnetic Resonance Imaging (MRI)).

Infarct volume measurements were performed by cerebral MRI in the first 24 hours after stroke and at 30 days after stroke onset for each patient.

Cerebral MRI can be used as a surrogate marker for the evaluation of the treatments effects, but the most important and reliable tool for this, remains the neurological evolution of the stroke patient. This study evaluated also the clinical evolution for each patient using NIHSS, Barthel Index, modified Rankin scale, made correlations with the MRI volume, and the results will be available soon.

The study objective

The aim of this trial was to test the hypothesis that patients randomized to Cerebrolysin would show an important reduction in stroke volume at 30 days from stroke onset, compared to patients randomized to placebo.

Additionally, it was hypothesized that Cerebrolysin, compared to a placebo, would show improved scores at 30 and 90 days post-stroke on tests measuring global neurological status, disability, quality of life and depression; the results of these functioning scales will be published in a subsequent manuscript.

Inclusion and exclusion criteria

The inclusion criteria were: (1) stroke onset 24 hours prior to the first infusion of the study drug; (2) stroke was ischemic in origin, supratentorial, radiologically confirmed (CT or MRI), and had a volume > 2 cc; (3) age between 18 and 80 years; (4) no significant pre-stroke disability (pre-stroke Modified Rankin Score of 0 or 1).

Patients with progressive or unstable stroke, pre-existing and active major neurological diseases, pre-existing and active (e.g., on chronic medication) major psychiatric diseases (such as major de-

pression, schizophrenia, bipolar disease, or dementia), advanced liver, kidney, cardiac, or pulmonary disease, a terminal medical diagnosis consistent with survival < 1 year, a substantial decrease in alertness at the time of randomization and an inability to give informed consent were excluded from the study. Furthermore, pregnant or lactating patients, patients with any condition that would represent a contraindication to Cerebrolysin (e.g., an allergy to Cerebrolysin) and patients participating in another therapeutic study of stroke or stroke recovery were excluded.

Study medication

Cerebrolysin was administered as a single daily dose of 30 ml diluted with physiological saline solution to a total volume of 250 ml as an intravenous infusion over a time period of one hour. An identical amount of physiological saline solution (250 ml) was administered as the placebo.

Study medication was administered once daily for 10 consecutive days. On the day of the baseline evaluation, the drug was administered as early as possible after patient randomization, always within a time window of 24 hours after stroke onset.

Blinding of investigators

Randomization envelopes were provided to a qualified person responsible for the preparation of the study medication at the clinical study center. This person was independent of all other study-specific procedures, including safety and efficacy assessments.

All investigators and study personnel were blinded to the treatment and used a randomized number for each sample.

In addition, the cerebral MRIs were performed in a private clinic and the radiologist responsible for the results was blind to the treatment group.

Stroke volume measurements

MRI stroke volumes were measured with the use of echo planar imaging on a 1.5-T General Electric Signa magnet. Multislice whole-brain diffusion-weighted imaging (DWI) was used to measure the stroke volume during the acute phase (in the first 24 hours after stroke). Parameters used were: 16 slices; repetition time was 8100 ms; echo time was 110 ms; slice thickness was 5 mm; gap was 2.5 mm; matrix was 128x128; field of view was 24 cm; *b* values were 0 and 741 s/mm^2.

DW images were acquired in the x, y, and z directions. Trace apparent diffusion coefficient maps were generated. The images were transferred to an image analysis software package (MRVision Software, MRVision Company), and the volumes were measured offline.

Stroke volume at 30 days after onset was measured using fluid-attenuated inversion recovery (FLAIR) images.

Statistics

Descriptive statistics, such as mean, standard deviation, median with interquartile interval, minimum and maximum values were used to summarize the data.

For determining whether the population was normally distributed, we used numerical and graphical methods, such as the Kolmogorov-Smirnov test, Q-Q plot, and skewness and kurtosis ±standard errors. The acceptable error threshold was $\alpha = 0.05$. The non-parametric Mann-Whitney, Wilcoxon Signed Ranks Test was used to evaluate the treatment effects on stroke volume and differences between treatment groups.

Differences in the mean age values between the two groups were compared using unpaired Student's t test.

Bivariate correlations for variables with non-Gaussian distribution were analyzed by the Spearman's rank correlation coefficient (rho). The significance tests were used to estimate the correlation coefficients (a probability value lower than 0,05 was considered significant). The Colton rules for empirical interpretation were also used.

Descriptive and inferential statistics were calculated using SPSS version 13 and StatSoft version 7.0.

RESULTS

As shown in Table 1, demographic data at baseline were similar in Cerebrolysin- and placebo-treated patients. There was no significant difference in the distribution of age between groups (independent Student's t test, mean difference = 2.8, t = 0.98, df = 58, p = 0.33).

TABLE 1. *Demographic data in Cerebrolysin- and placebo-treated groups*

Group	Cerebrolysin (N=30)	Placebo (N=30)
Age (years)		
Mean	62,90	65,70
Median	63,0	69,0
SD	10,99	11,23
Minimum	33	38
Maximum	79	79
Sex		
Male	19	20
Female	11	10

A summary of the data related to the initial and final stroke volumes in each group are presented in Table 2.

***TABLE 2**. Descriptive statistics of stroke volumes in Cerebrolysin – and placebo-treated groups*

	Cerebrolysin		Placebo	
	Initial stroke volume (cc)	Final stroke volume (cc)	Initial stroke volume (cc)	Final stroke volume (cc)
Median (inter-uartile intervals: Q1-Q3)	18,5 (4,48-8,32)	5,47 (2,15-12,60)	12,49 (5,41-29,65)	6,21 (2,35-13,67)
Minimum	2,15	0,55	2,31	0,42
Maximum	236,05	63,46	289,52	85,10

No significant difference in the initial stroke volume was found between the groups. (Mann–Whitney test, U = 439, p = 0.871).

A greater variability in the initial stroke volume was present in the Cerebrolysin group (4,48-8,32) versus the placebo group (5,41-29,65). The final stroke volume values were relatively homogeneous between Cerebrolysin (2.15 to 12.60) and placebo (2.35 to 13.67) groups.

A significant reduction of stroke volume at 30 days was observed in both the Cerebrolysin (Wilcoxon Signed Ranks test, Z = -4,76, p < 0.001) and placebo-treated groups (Wilcoxon Signed Ranks test, Z = -4,45, p < 0.001), as shown in Figure 1.

Although it was not statistically significant, evidence suggests that the Cerebrolysin-treated group had a better response in stroke volume reduction compared to the placebo group (Mann Whitney test, U = 426, p = 0.723, mean rank, 29.70 vs. 31.30).

No correlations were found between age and the initial stroke volume in the Cerebrolysin group (Spearman correlation coefficient, ρ = -0.211, p = 0.264) or the placebo group (Spearman correlation coefficient, ρ = -0.281, p = 0.132).

No correlations were found between age and the final stroke volume in the Cerebrolysin group (Spearman correlation coefficient, ρ = -0.186, p = 0.324) or the placebo group (Spearman correlation coefficient, ρ = -0.149, p = 0.432).

No association was found between sex and the initial stroke volume, regardless of the treatment group (Mann-Whitney test, p > 0.05).

DISCUSSION

This study has some limitations, including a small sample size, heterogeneity of the demographic data, and heterogeneity of the stroke subtypes.

Age and initial DWI stroke volume are independent markers used to predict to functional outcome (16,18). In our study, correlations between the age of the patient and the initial and final stroke volumes did not support age as a predictor for the evolution of the size of ischemic damage.

In a retrospective study, Gaudisnki et al. (23) described the evolution of ischemic stroke lesions on MRI. The authors showed that even if ischemic le-

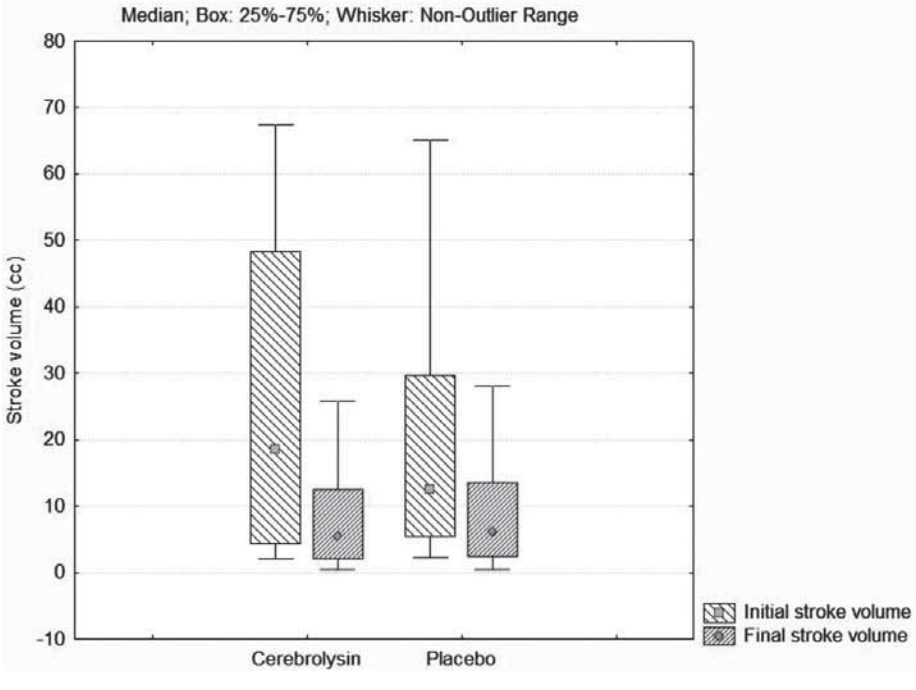

***FIGURE 1.** Reduction of stroke volume in Cerebrolysin- and placebo-treated groups*

sions continued to evolve between 5 and 90 days, lesion volume approached final stroke volume at 30 days. The authors proposed that lesion volumes measured at 30 days could be used as an objective surrogate marker for use in early phase clinical trials (23). Typically, DWI lesion volumes increase during the first 3 days after stroke onset and slowly decrease in size during the subsequent 3 to 4 weeks (24). The initial increase of ischemic stroke volume is due to the development of vasogenic edema and the expanding of ischemia into surrounding tissue (24,25). The reduction of stroke volume at 4 weeks (measured with MRI) can be explained by the resolution of edema, reperfusion of salvageable tissue, sulcal atrophy, ventricular enlargement, hypodense cavities, or neuroplasticity and neurorecovery processes (24,26). Neuroplasticity represents the brain's ability to change existent structures and is responsible for brain reorganization after injury (6). Neuroplasticity involves the activation of existing but silent connections, synaptogenesis, dendritic arborization and new nervous cell production (27,28). Neuroplasticity can be enhanced by administration of neurotrophic factors (6,29). Due to its unique composition consisting of active fragments of neurotrophic factors, Cerebrolysin is able to confer neuroprotection and to stimulate neuroplasticity, thereby enhancing the neurorecovery process (10,21,22).

Although the results were not statistically significant between the drug and placebo groups, an meaningful stroke volume reduction was found in favor of Cerebrolysin-treated patients (Mann Whitney test, U = 426, p = 0,723, mean rank, 29.70 vs. 31,30). This effect can be explained by the neuroprotective and neuroplastic effects of the compound Cerebrolysin (10). Similar positive results (45.4% versus 43.6% in the placebo-group, $p < 0.05$) were reported by Shamalov et al. (30). The authors included forty-seven patients with ischemic stroke, aged 45-85 years, where the treatment with Cerebrolysin (50 ml/day for 10 days) was initiated in the first 12 hours after stroke (30). Skvortsova et al. compared the effects of two different doses of Cerebrolysin (10 ml/day and 50 ml/day for 10 consecutive days) versus placebo (31). Thirty-six patients with acute ischemic stroke, aged 45-85 years, were included. The study medication was introduced in the first 12 hours after stroke and a significant reduction in the stroke volume for both Cerebrolysin and placebo groups was measured using MRI on day 3 ($p < 0.05$ vs. placebo) (31).

The lack of statistical significance in our study can be explained by the small number of patients included in the study and the heterogeneity of stroke volume and stroke subtypes.

CONCLUSIONS

Despite study limitations, the data suggests a positive effect of Cerebrolysin treatment on the reduction of ischemic stroke volume. This observation can be attributed to the dual mechanism of action of Cerebrolysin. The compound not only has neuroprotective effects that are relevant to stroke treatment in the acute stages, but it also promotes recovery after stroke by potentially enhancing neuroplasticity, based on data from animal models. These recovery-promoting effects of Cerebrolysin support an extended time window of opportunity for clinical treatment, compared to the time window previously suggested for neuroprotective effects.

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Conflicts of interests

The authors declare no conflicts of interest.

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