A Retrospective, Multi-Center Cohort Study Evaluating the Severity-Related Effects of Cerebrolysin Treatment on Clinical Outcomes in Traumatic Brain Injury

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Abstract: Traumatic brain injury (TBI) is a leading cause of death and disability for which there is currently no effective drug therapy available. Because drugs targeting a single TBI pathological pathway have failed to show clinical efficacy to date, pleiotropic agents with effects on multiple mechanisms of secondary brain damage could represent an effective option to improve brain recovery and clinical outcome in TBI patients. In this multicenter retrospective study, we investigated severity-related efficacy and safety of the add-on therapy with two concentrations (20 ml/day or 30 ml/day) of Cerebrolysin (EVER Neuro Pharma, Austria) in TBI patients. Adjunctive treatment with Cerrebrolysin started within 48 hours after TBI and clinical outcomes were ranked according to the Glasgow Outcome Scale and the Modified Rankin Disability Score at 10 and 30 days post-TBI. Analyses of efficacy were performed separately for subgroups of patients with mild, moderate or severe TBI according to Glasgow Coma Scale scores at admission. Compared to standard medical care alone (control group), both doses of Cerebrolysin were associated with improved clinical outcome scores at 10 days post-TBI in mild patients and at 10 and 30 days in moderate and severe cases. A dose-dependent effect of Cerebrolysin on TBI recovery was supported by the dose-related differences and the significant correlations with treatment duration observed for outcome measures. The safety and tolerability of Cerebrolysin in TBI patients was very good. In conclusion, the results of this large retrospective study revealed that early Cerebrolysin treatment is safe and is associated to improved TBI outcome.

Keywords: Cerebrolysin, disability, functional recovery, neuroprotection, clinical outcome, traumatic brain injury.

INTRODUCTION

The clinical management of traumatic brain injury (TBI) remains a challenging issue, and further research on neuroprotective and recovery therapies is needed. An improved understanding of the pathophysiological mechanisms of TBI, particularly the evolution of secondary damage, could provide targets for novel treatment approaches [1, 2]. One recurrent problem is the translation of promising results from experimental animal models to successful clinical therapies. The complexity of TBI sequelae requires a multifactorial approach for improved neurological recovery. Pharmacological intervention represents an increasingly useful therapeutic asset, particularly with the development of multimodal drugs.

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A continuous brain response, which has been defined as endogenous defense activity (EDA), occurs after an acute brain lesion [3]. The EDA consists of two major sequences: an immediate sequence of neuroprotection that decreases neurological damage and its consequent impairment, and a later sequence of neurorepair. Neurorecovery is the functional consequence of the neurorepair processes and represents positive and clinically relevant results. Neurorecovery is supported by neurotrophicity, neuroprotection, neuroplasticity and neurogenesis, the fundamental biological processes that generate EDA [3]. Several etiological agents trigger common pathophysiological processes that may overcome EDA and generate different neurological diseases with an acute or a chronic course. The molecular cascades triggered by these pathophysiological mechanisms are similar, regardless of the etiological diversity or clinical polymorphism, and these cascades can lead to necrotic or apoptotic-like cell death [4]. Modulating these pathological cascades at different points with multimodal pleiotropic neuroprotective drugs within an effective timeframe may attenuate cell death and generate pharmacological neuroprotection and recovery that supports EDA.

The multimodal effect of a drug is based on its capacity to link immediate acute neuroprotection with long-term reparatory processes (e.g., neurotrophicity, neuroplasticity and neurogenesis), which is similar to the actual sequence of endogenous post-lesional regulation. Pharmacological mechanisms that closely mimic endogenous biological mechanisms without blocking secondary reparatory neuroplasticity, which is the most important driver of neurorecovery, may represent the future of pharmacotherapy for brain protection and recovery. Therefore, neurotrophic factors and neurotrophic-like molecules are promising candidates for TBI treatment [2, 5]. Cerebrolysin is the only clinically available multimodal pleiotropic neuroprotective drug with active low molecular weight fragments of neurotrophic factors that can pass the blood-brain barrier. Cerebrolysin is produced using a standardized enzymatic breakdown of purified, lipid-free brain proteins. Cerebrolysin has a low molecular weight (<10 kDa) and contains biologically active fragments of neurotrophic neuropeptides and free amino acids.

Cerebrolysin mimics the action of endogenous neurotrophic factors and exerts neuroprotective and neurorestorative effects *in vitro* [6-8]. Cerebrolysin protects against cytoskeletal degradation, reduces edema formation, enhances neurogenesis and lowers the mortality rate in animal models of hypoxia and ischemia. Cerebrolysin reduced infarct volume and improved neurological outcome in a rat model of acute focal ischemia [9]; it also significantly improved the neurological outcome compared with placebo in a rat model of middle cerebral artery occlusion when treatment was initiated up to 48 h after stroke onset [10, 11]. Cerebrolysin demonstrated positive results in a meta-analysis of clinical trials examining cytoprotection after stroke [12].

In animal models of TBI, Cerebrolysin reduced blood-brain barrier (BBB) leakage, attenuated brain edema and neuronal damage, and improved functional recovery [13-15]. Importantly, the window of opportunity for effective Cerebrolysin treatment after brain trauma may be wide due to its effects on neuroplasticity. Previous clinical studies reported positive effects for Cerebrolysin on clinical outcome and on brain bioelectrical activity, cognitive performance, and functional recovery during rehabilitation in TBI patients, which may result in a shorter hospitalization time and a better long-term outcome [2, 16-21]. Therefore, Cerebrolysin appears to have the appropriate pharmacological profile to improve clinical recovery in TBI patients.

The severity-related effects of Cerebrolysin on TBI clinical outcome have not been investigated. Thus, and taken into account experimental and clinical experience with the drug, we postulated that the administration of Cerebrolysin neuropeptides as adjunctive therapy would be well tolerated and would foster short-term recovery independently of TBI severity. In order to test our hypothesis, in the present, study we examined whether adding Cerebrolysin to the standard medical care of patients with mild, moderate and severe head injuries improved functional recovery and clinical outcome. We also documented the safety profile of Cerebrolysin in these patients.

MATERIALS AND METHODS

Patients

A total of 7,769 adult patients with TBI who were admitted to 10 Romanian neurosurgery departments between 2005-2010 were included in this retrospective study. All of the patients were managed according to standard guidelines on medical-surgical care of TBI [22-24], but some (1,618) received Cerebrolysin® (EVER Neuro Pharma, Austria) as an adjuvant therapy beginning within the first 48 hours after TBI. The following factors were used as retrospective selection and inclusion criteria: age over 18 years, mild to severe closed head injury according to the Glasgow Coma Scale (GCS), admission within 48 hours of TBI onset, discharged or follow-up at 10 days and follow-up at 30 days. The exclusion criteria included the following conditions: life-threatening multiple trauma, other associated severe conditions, epilepsy, concomitant stroke, pregnancy, lactation, concomitant medication with neuroprotective or nootropic effects (e.g., citicoline, amantadine, memantine, erythropoietin, piracetam, pramiracetam, pyritinol, and meclosulfonate), vasoactive drugs (naftidrofuryl, cinnarizine, flunarizine, nimodipine, nicergoline, pentoxifylline, dihydroergotoxine, vinpocetine, vincamine and gingko biloba) or psychotropic drugs (antidepressants, neuroleptics, sedatives, hypnotics, and CNS stimulants).

Cerebrolysin is approved for use in humans in Romania and was always administered at the hospital as add-on treatment upon consent by the patient and/or the responsible caregiver, Decision on the administration of Cerebrolysin was taken in all instances by the medical team in charge of the patient's care and according to their clinical experience and professional judgment. Therefore, this retrospective study reflects the state of the art of TBI management in the country, with adherence to the principles of the Declaration of Helsinki and following recommendations of the international guidelines on the standard medical-surgical management of TBI [22-24].

Study Design, Treatment Regimen and Evaluation Procedures

All of the patients were evaluated at admission using standardized diagnosis guidelines and a unique protocol. The patients' records were queried, and data were collected repeatedly, addressing the clinical status at admission, day 10 after TBI, and day 30 after TBI. The general data (gender, age, etiology, medical history, concomitant medication, GCS score, clinical neurological examination, computerized tomography (CT) result, and surgical intervention) were collected from the medical records at admission. The patients were ranked on the Glasgow Outcome Scale (GOS) and Modified Rankin Disability Score (RDS) 10 and 30 days post-TBI based on medical records. The safety assessments, such as adverse events, vital signs, laboratory tests and clinical examinations, were extracted from the patients' medical records. Efficacy and safety evaluations were done by personnel not involved in any treatment decision concerning the evaluated patients. The Cerebrolysin-treated patients were grouped into 2 different drug regimens (20 ml/day or 30 ml/day, administered through i.v. infusion), each of which was compared with the control group. Of the patients on Cerebrolysin treatment, 1,142 received 20 ml/day, and 476 were treated with the 30 ml/day dose. The duration of the Cerebrolysin treatment varied from 1 to 30 days, and the median treatment duration was 10 days.

Study Objectives

This retrospective study investigated the effects of add-on Cerebrolysin treatment compared with standard medical care alone on clinical outcome in subgroups of mild, moderate and severe TBI patients at 10 and 30 days post-TBI and evaluated the safety of Cerebrolysin in these patients.

Statistics

The statistical comparisons were performed using SPSS Statistics 19 (IBM). The comparisons were stratified according to admission GCS score subgroups (severe = 3-8, moderate = 9-12 and mild TBI = 13-15). Group comparisons were done by chi-square test, and one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc tests, as appropriate. The *t*-test for equality of means, and the Pearson linear correlation test were also used. The results are expressed as the means ± standard deviation (SD) in the text and tables and as the means ± standard error (SE) in the figures. A threshold of $p<0.05$ was considered significant. We used parametrical statistical tests, as the kurtosis of the outcome parameters in question (GOS at 10 and 30 days, RDS at 10 and 30 days) was >1.96, the standard error of kurtosis.

Consent in writing was obtained from all patients and/or the responsible caregivers, and the present investigation was reviewed and approved by the Institutional Review Board and Ethical Committee of the Iuliu Haţieganu University of Medicine and Pharmacy (Cluj Napoca, Romania).

RESULTS

Demographic and Baseline Clinical Data

The mean age of the Cerebrolysin treated patients was 49.4 ± 19.0 (range 18 to 95) years, which was significantly higher ($p<0.001$) than that of the control patients (46.0 ± 19.4, range 18 to 96 years). Gender distribution was also different in control and Cerebrolysin groups, representing males 68.8% and 73% of the cases, repectively (Table **1**). Significant differences were also noted between study groups for the distribution of TBI etiologies (Table **1**). Car crash was more frequent in the control group; whereas other traffic accidents, falls from ground level and loss of consciousness showed a higher frequency in the Cerebrolysin group. The clinical neurological symptoms-signs and cranial CT scan findings at admission are summarized in Tables **2** and **3**, respectively. A higher percentage of patients presented a comatose state (28.9%), intracranial hypertension (38.4%), hemiparesis (19.2%) or memory disturbances (8.3%) in the Cerebrolysin group than in the control group (12.4%, 21.3%, 7.6%, and 4.8%, respectively, $p<0.001$ for all comparisons). Conversely, headache, confusion and aphasia were recorded more frequently in the control group (Table **2**). Consistent with differences in the clinical picture, the CT was normal in a lower percentage of patients in the Cerebrolysin group than in the control group (24.3% *vs* 46.9%, respectively, $p<0.001$) (Table **3**). Brain contusion and dilaceration, extradural and subdural hematoma, cerebral edema, cranial fracture, and intraparenchimal hematoma were all more prevalent in the Cerebrolysin group than in the control group (Table **3**). Brain damage severity, as determined *via* the GCS score at admission, is presented in Table **4**. The average GCS score at admission was significantly lower ($p<0.001$) in the Cerebrolysin group (10.9 ± 3.8) than in the control group (13.2 ± 3.3). Within the Cerebrolysin group, 745 patients (46.0%) were classified as having mild TBI, 406 patients (25.1%) were classified as having moderate TBI, and 467 patients (28.9%) were classified as having severe TBI; whereas 4,787 (77.8%), 604 (9.8%) and 760 (12.4%) patients had mild, moderate or severe TBI, respectively, in the control group. This distribution reflects a more marked clinical severity ($p<0.001$) in Cerebrolysin-treated patients than in control group cases (Table **4**). Surgical treatment was performed in 437 patients (27.0%) in the Cerebrolysin group compared with 1,526 patients (24.8%) in the control group (no significant difference). The proportion of patients with moderate or severe TBI was higher in the Cerebrolysin group than in the control group; thus, analyses of efficacy were performed separately for the subgroups of mild, moderate and severe TBI patients.

Efficacy of Cerebrolysin Treatment in Mild TBI

In the mild TBI subgroup, 4,787 patients were treated according to the standard medical care alone, and 745 patients received additional treatment with Cerebrolysin (615 patients received 20 ml/day and 130 patients received

Table 1. Demographic and etiological characteristics of the TBI study groups.

Parameter		Control	Cerebrolysin	Significance (t-Test; Chi-Square)
		Mean ± SD	Mean ± SD	
Age (years)		46.0 ± 19.4	49.4 ± 19.0	p< 0.001
		N (%)	N (%)	
Gender Male/Female		4.233/1.918 (68.8/32.2)	1.182/436 (73.0/27.0)	X^2: 10.88; df:1; p<0.01
TBI etiology	Car crash	3.062 (49.8)	664 (41.0)	X^2: 124.23; df: 6; p<0.001
	Other traffic accidents	110 (1.8)	83 (5.1)	
	Fall from ground level	1.131 (18.4)	345 (21.3)	
	Fall from a high level	641 (10.4)	195 (12.1)	
	Aggression	1.107 (18.0)	270 (16.7)	
	Loss of consciousness	31 (0.5)	30 (1.9)	
	Unspecified conditions	69 (1.1)	31 (1.9)	
Total number of cases		6.151 (100)	1.618 (100)	

Group means were compared by t-test and differences between groups for the distribution of the indicated parameters were analyzed by using the chi-square test.

Table 2. Clinical neurological symptoms and signs in each study group at admission.

Symptom-Sign	Control N (%)	Cerebrolysin N (%)	Significance (Chi-Square)
Coma	760 (12.35)	467 (28.86)	X^2: 262.47; df:1; p<0.001
Intracranial Hypertension	1,213 (21.34)	622 (38.44)	X^2: 248.90; df:1; p<0.001
Hemiparesis	470 (7.64)	311 (19.22)	X^2: 189.99; df:1; p<0.001
Headache	3,642 (59.20)	567 (35.04)	X^2: 301.37; df:1; p<0.001
Memory disturbance	295 (4.79)	135 (8.34)	X^2: 13.12; df:1; p<0.001
Confusion	440 (7.15)	75 (4.63)	X^2: 30.84; df:1; p<0.001
Somnolence	249 (4.08)	67 (4.14)	X^2:0.03; df:1; ns
Agitation	155 (2.51)	45 (2.78)	X^2: 0.35; df:1; ns
Aphasia	192 (3.12)	35 (2.16)	X^2: 4.15; df:1; p< 0.05
Sensory deficit	77 (1.25)	29 (1.79)	X^2: 2.78; df:1; ns

Differences between groups for the distribution of the indicated parameters were analyzed by using the chi-square test. The percentages refer to each particular group of patients.

Table 3. Cranial CT scan findings in each study group at admission.

CT Result	Control N (%)	Cerebrolysin N (%)	Significance (Chi-Square)
Normal	2,882 (46.85)	393 (24.28)	X^2: 267.49; df:1; p<0.001
Contusion	1,333 (21.67)	699 (43.20)	X^2: 307.45; df:1; p<0.001
Dilaceration	222 (3.60)	171 (10.56)	X^2: 129.19; df:1; p<0.001
Extradural hematoma	330 (5.36)	151 (9.33)	X^2: 34.72; df:1; p<0.001
Subdural hematoma	887 (14.42)	288 (17.79)	X^2: 11.40; df:1; p<0.01
Subarachnoid hemorrhage	397 (6.45)	109 (6.73)	X^2: 0.17; df:1; ns
Cerebral edema	528 (8.58)	170 (10.50)	X^2: 5.79; df:1; p<0.05
Fracture	1,361 (22.12)	449 (27.75)	X^2: 22.67; df:1; p<0.001
Intraparenchymal hematoma	25 (0.40)	16 (0.98)	X^2: 8.28; df:1; p<0.01

CT: Computerized Tomography. Differences between groups for the distribution of the indicated parameters were analyzed by using the chi-square test. The percentages refer to each particular group of patients.

Table 4. Particular GCS scores and GCS score-related severity in each study group at admission.

GCS Score	Control	Cerebrolysin	Significance
	N (%)	N (%)	
3	132 (2.1)	47 (2.9)	
4	177 (2.9)	97 (6.0)	
5	63 (1.0)	60 (3.7)	
6	154 (2.5)	79 (4.9)	
7	123 (2.0)	96 (5.9)	
8	111 (1.8)	88 (5.4)	
9	101 (1.6)	56 (3.5)	
10	137 (2.2)	98 (6.1)	
11	107 (1.7)	88 (5.4)	
12	259 (4.2)	164 (10.1)	
13	250 (4.1)	157 (9.7)	
14	789 (12.8)	259 (16.0)	
15	3,748 (60.9)	329 (20.3)	
Average GCS Score	**Mean±SD**	**Mean±SD**	**Significance (t-Test)**
	13.20±3.26	10.94±3.76	F: 200.57; df:7767; p<0.001
GCS-Related Severity:	**N (%)**	**N (%)**	**Significance (Chi-Square)**
3-8 (severe)	760 (12.4)	467 (28.9)	X^2: 632.57; df:2; p<0.001
9-12 (moderate)	604 (9.8)	406 (25.1)	
13-15 (mild)	4,787 (77.8)	745 (46.0)	
Total number of cases	6,151 (100)	1,618 (100)	

GCS: Glasgow Coma Scale. Differences between groups for the distribution of the indicated parameters (GCS score; GCS-related severity) were analyzed by using the chi-square test. The percentages refer to each particular group of patients.

30 ml/day). The GOS mean scores were significantly higher (better outcome) in both the 20 ml/day and 30 ml/day Cerebrolysin treatment groups (4.80 ± 0.47 and 4.86 ± 0.43, respectively) than in the control group (4.62 ± 0.56, p<0.001, Fig. **1**) at 10 days post-TBI. No significant differences were observed between the GOS mean scores in the 20 ml/day and 30 ml/day Cerebrolysin groups at 10 days post-TBI. The GOS mean scores were not significantly different between the Cerebrolysin (4.93 ± 0.37 for 20 ml/day and 4.90 ± 0.41 for 30 ml/day) and control groups (4.93 ± 0.36) at 30 days post-TBI. Similar to the GOS results, the RDS mean scores were significantly lower (better outcome) in both the 20 ml/day and 30 ml/day Cerebrolysin treatment groups (0.68 ± 0.93 and 0.54 ± 1.00, respectively) than in the control group (0.95 ± 1.1, p<0.001, Fig. **2**) at 10 days post-TBI. The RDS mean scores were not significantly different between the Cerebrolysin (0.26 ± 0.70 for 20 ml/day and 0.26 ± 0.83 for 30 ml/day) and control groups (0.25 ± 0.72) at 30 days post-TBI. The Cerebrolysin treatment duration was slightly but significantly correlated with the GOS (Pearson coefficient 0.096, p<0.001) and RDS (Pearson coefficient -0.065, p<0.001) scores at 10 days but not at 30 days post-TBI.

The average GCS scores at admission were compared to assess the differences in clinical severity at baseline between the treatment groups. Mild TBI patients treated with 20 ml/day and 30 ml/day of Cerebrolysin had significantly lower average GCS scores (14.20 ± 0.77 and 14.36 ± 0.78, respectively) at admission than the control patients (14.73 ± 0.55, p<0.001).

Efficacy of Cerebrolysin Treatment in Moderate TBI

In the moderate TBI subgroup, 604 patients received the standard medical care alone and 406 patients received the standard medical care plus Cerebrolysin (314 patients received 20 ml/day and 92 patients received 30 ml/day). At 10 days, the GOS mean scores were significantly higher in both the 20 ml/day and 30 ml/day Cerebrolysin treatment groups (4.12 ± 0.99 and 4.40 ± 0.81, respectively) than in the control group (3.77 ± 0.98, p<0.001, Fig. **3**). The GOS mean scores at this time point were also significantly higher in the 30 ml/day Cerebrolysin group than in the 20 ml/day Cerebrolysin group (p<0.05, Fig. **3**), which suggests a dose-dependent effect. Significant treatment differences were maintained at 30 days post-TBI, with the GOS mean scores higher in the Cerebrolysin-treated patients (4.58 ± 1.01 for 20 ml/day and 4.76 ± 0.78 for 30 ml/day) than in the control group (4.23 ± 1.13) (Fig. **3**). Similarly to the GOS results, the RDS mean scores were significantly lower in both the

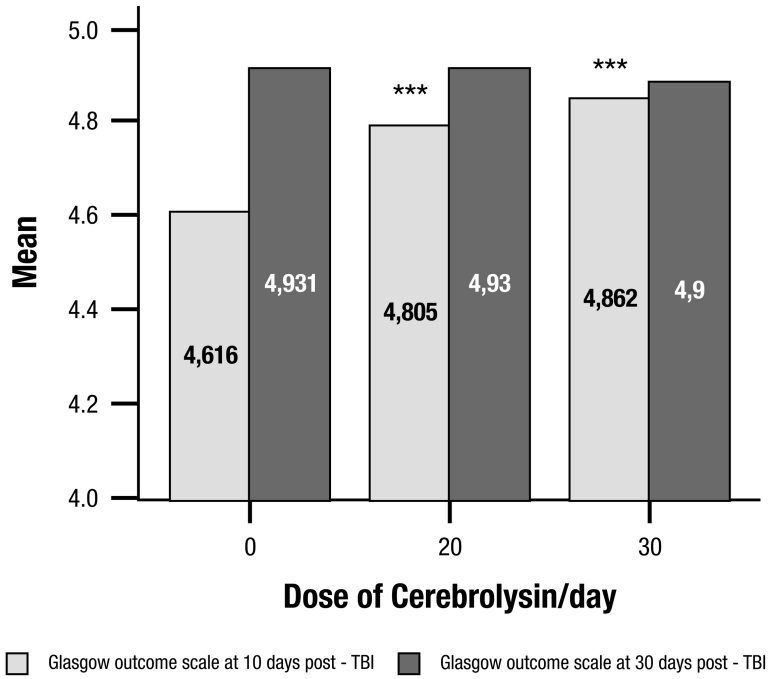

Fig. (1). GOS scores at 10 and 30 days post-TBI in the treatment groups of mild TBI patients. The control group is designated as Cerebrolysin 0 ml/day. At 10 days post-TBI, the average GOS score was significantly higher in both the 20 ml and 30 ml Cerebrolysin treatment groups compared with the control group. *** = $p<0.001$ for the Bonferroni post-hoc test at 10 days compared with the controls.

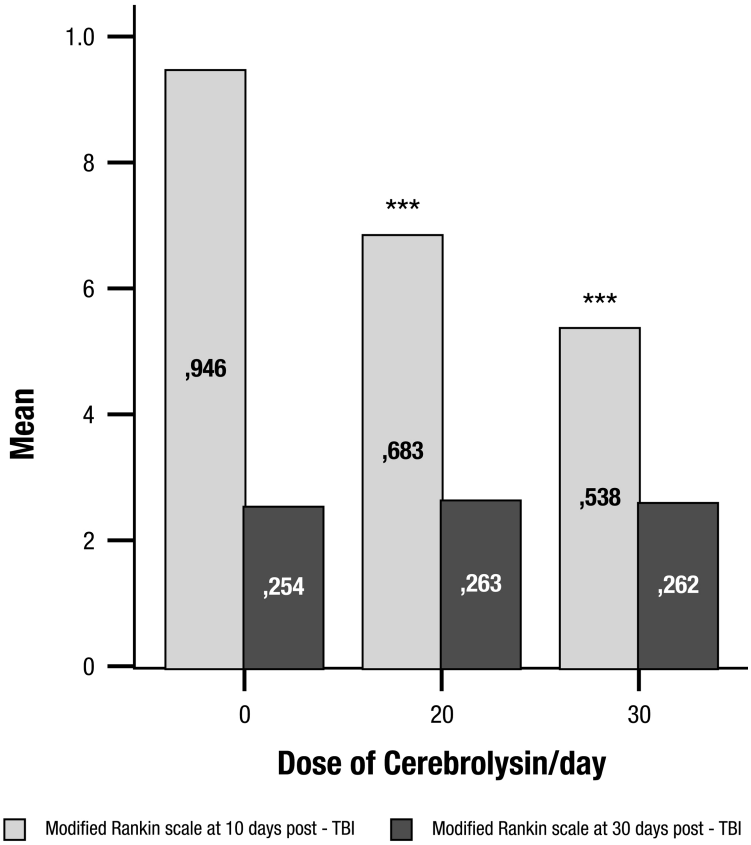

Fig. (2). RDS scores at 10 and 30 days post-TBI in the treatment groups of mild TBI patients. The control group is designated as Cerebrolysin 0 ml/day. At 10 days post-TBI, the average RDS score was significantly lower in both the 20 ml and 30 ml Cerebrolysin treatment groups compared with the control group. *** = $p<0.001$ for the Bonferroni post-hoc test at 10 days compared with the controls.

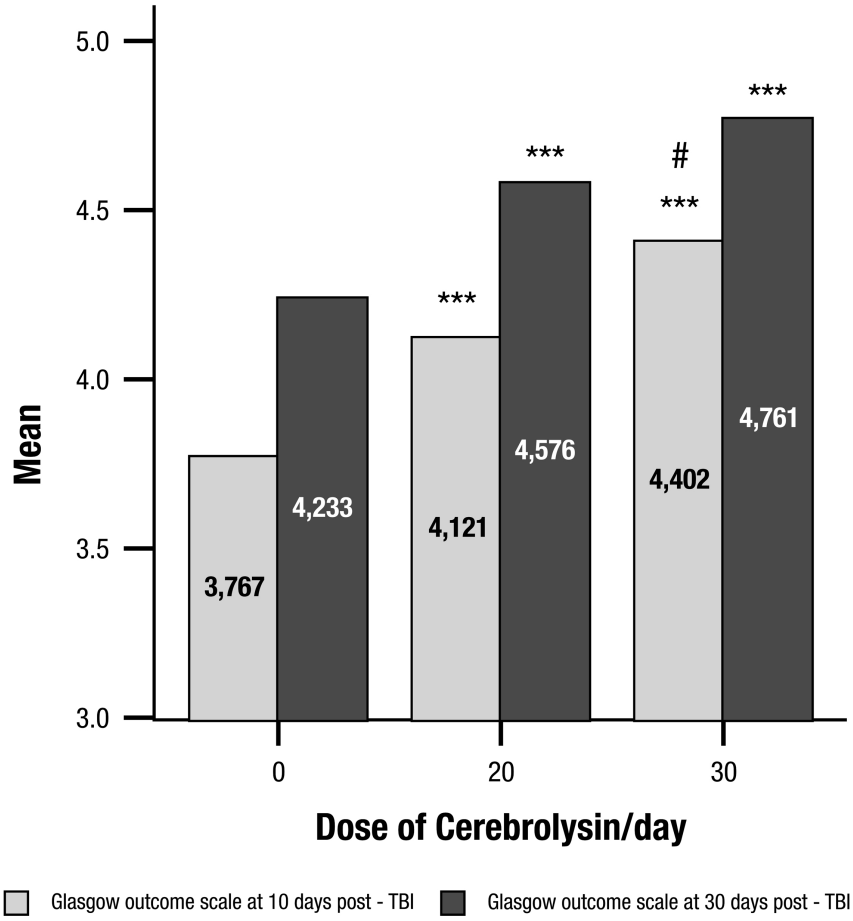

Fig. (3). GOS scores at 10 and 30 days post-TBI in the treatment groups of moderate TBI cases. The control group is designated as Cerebrolysin 0 ml/day. At 10 and 30 days post-TBI, the average GOS score was significantly higher in both 20 ml and 30 ml Cerebrolysin treatment groups compared to the control group. At 10 days, the average GOS score was significantly higher in the 30 ml Cerebrolysin treatment group compared with the 20 ml Cerebrolysin treatment group. *** = $p<0.001$ for the Bonferroni post-hoc test at 10 and 30 days compared with the controls; # = $p<0.05$ for Cerebrolysin 30 ml/day compared with Cerebrolysin 20 ml/day at 10 days.

20 ml/day and 30 ml/day Cerebrolysin treatment groups (2.01 ± 1.49 and 1.79 ± 1.21, respectively) than in the control group (2.61 ± 1.45, $p<0.001$, Fig. **4**) at 10 days post-TBI. The RDS mean scores were also significantly lower in the Cerebrolysin-treated groups (0.99 ± 1.65 for 20 ml/day and 0.70 ± 1.25 for 30 ml/day) than in the control group (1.79 ± 1.74, $p<0.001$, Fig. **4**) at 30 days post-TBI. However, the difference in the mean scores between the 30 ml/day and 20 ml/day Cerebrolysin treatment groups did not reach statistical significance at the 10-day or 30-day time points. The duration of Cerebrolysin treatment correlated with GOS and RDS scores at 10 days (Pearson coefficients of 0.202 and -0.203, respectively, $p<0.001$ for both scores) and at 30 days (Pearson coefficients of 0.199 and -0.247, respectively, $p<0.001$ for both scores) post-TBI in the subgroup of moderately severe patients.

Like for the mild TBI subgroup, the average GCS scores at admission were compared to assess the differences in clinical severity at baseline between the treatment groups of moderate TBI patients. No significant differences in the average GCS scores at baseline were observed between the groups (Cerebrolysin 20 ml/day: 10.98 ± 1.06; Cerebrolysin 30 ml/day: 10.58 ± 1.14; control group: 10.87 ± 1.14).

Efficacy of Cerebrolysin Treatment in Severe TBI

In the severe TBI subgroup, 760 patients received only the standard medical care and 467 patients received additional treatment with Cerebrolysin (213 patients received 20 ml/day and 254 patients received 30 ml/day). The GOS mean scores were significantly higher in both the 20 ml/day (2.85 ± 1.25, $p < 0.01$) and the 30 ml/day (3.27 ± 1.14, $p<0.001$) Cerebrolysin treatment groups than in the control group (2.53 ± 1.32, Fig. **5**) at 10 days post-TBI. At this time point, the GOS mean scores were also significantly higher in the 30 ml/day Cerebrolysin group than in the 20 ml/day Cerebrolysin group ($p<0.01$, Fig. **5**), which suggests a dose-dependent effect. Significant treatment differences were maintained at 30 days post-TBI; the GOS scores were higher in the Cerebrolysin-treated patients (3.16 ± 1.65 for 20 ml/day, $p < 0.01$ and 3.51 ± 1.50 for 30 ml/day, $p<0.001$) than in the control group (2.78 ± 1.60, Fig. **5**). Consistent with the GOS results, the RDS mean scores were

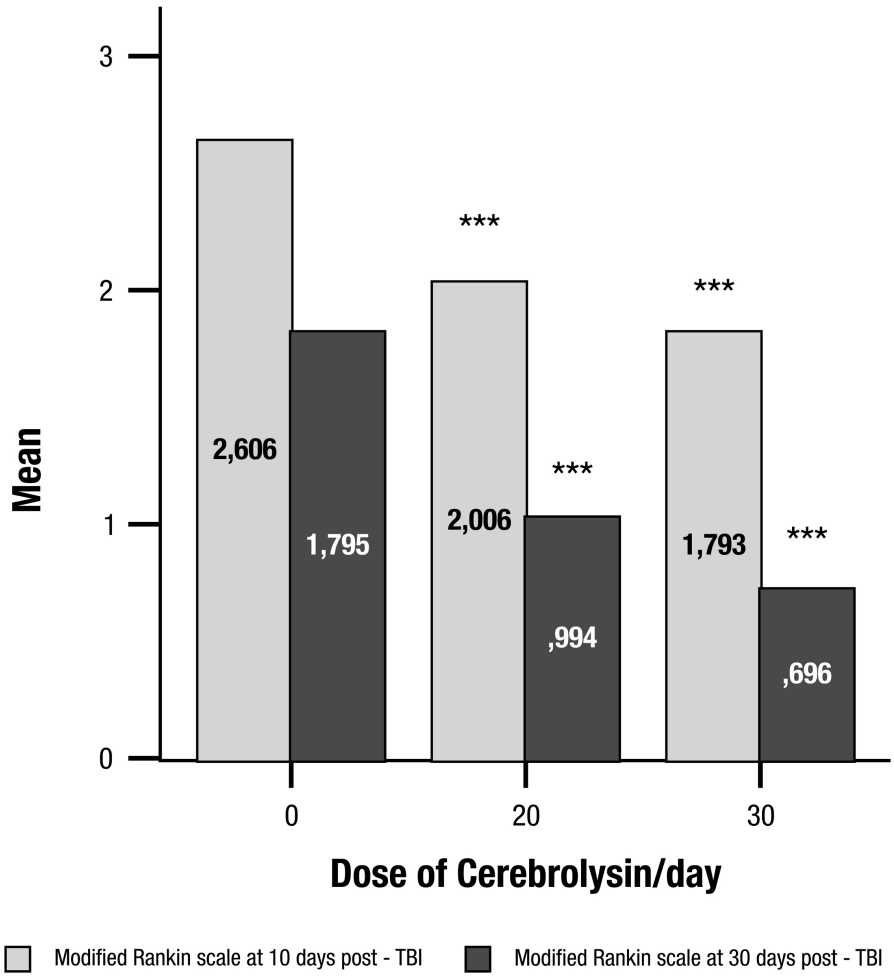

Fig. (4). RDS scores at 10 and 30 days post-TBI in the treatment groups of moderate TBI cases. The control group is designated as Cerebrolysin 0 ml/day. At 10 and 30 days post-TBI, the average RDS score was significantly lower in both the 20 ml and 30 ml Cerebrolysin treatment groups compared with the control group. *** = $p<0.001$ for the Bonferroni post-hoc test at 10 and 30 days compared with the controls.

significantly lower in both the 20 ml/day and 30 ml/day Cerebrolysin treatment groups (3.96 ± 1.60, $p < 0.01$ and 3.59 ± 1.45, $p<0.001$, respectively) than in the control group (4.34 ± 1.64, Fig. **6**) at 10 days post-TBI. The RDS mean scores remained significantly reduced in the Cerebrolysin-treated groups (3.38 ± 2.15, $p<0.05$ for 20 ml/day and 2.95 ± 1.98, $p<0.001$ for 30 ml/day) compared with the control group (3.81 ± 2.10, Fig. **6**) at 30 days post-TBI. The mean RDS scores in the 30 ml/day Cerebrolysin group were also significantly lower than those of the 20 ml/day Cerebrolysin group ($p<0.05$, Fig. **6**) at the 10-day time point. The duration of the Cerebrolysin treatment was found to significantly correlate with the GOS and RDS scores at 10 days (Pearson coefficients of 0.231 and -0.184, respectively, $p<0.001$ for both scores) and 30 days (Pearson coefficients of 0.222 and -0.188, respectively, $p<0.001$ for both scores) post-TBI.

The average GCS scores at admission were significantly higher ($p<0.05$) in the 30 ml/day Cerebrolysin group (5.98 ± 1.61) than in the 20 ml/day Cerebrolysin group (5.45 ± 1.68) or the control group (5.38 ± 1.71).

Safety of Cerebrolysin Treatment in TBI

No significant treatment-related differences were observed in the safety analyses, except for diarrhea, which was reported more frequently in Cerebrolysin-treated patients ($p<0.01$). Table **5** includes a group comparison of the distribution of the most frequent adverse events reported in patients considered for this study. The prevalence of these adverse events was rather low in all treatment groups, and only constipation, pyrexia, hypertension, insomnia, and urinary tract infection were reported by more than 5% of the patients on standard medical care and/or the patients treated with Cerebrolysin. In addition, there were no reports of anaphylactic shock or epileptic seizures after the administration of Cerebrolysin, and the occurrence of allergic reactions was very low in all treatment groups, and even lower in Cerebrolysin-treated patients. The lack of safety concerns observed in this retrospective study is consistent with safety results of the prospective clinical studies performed with Cerebrolysin in TBI [2].

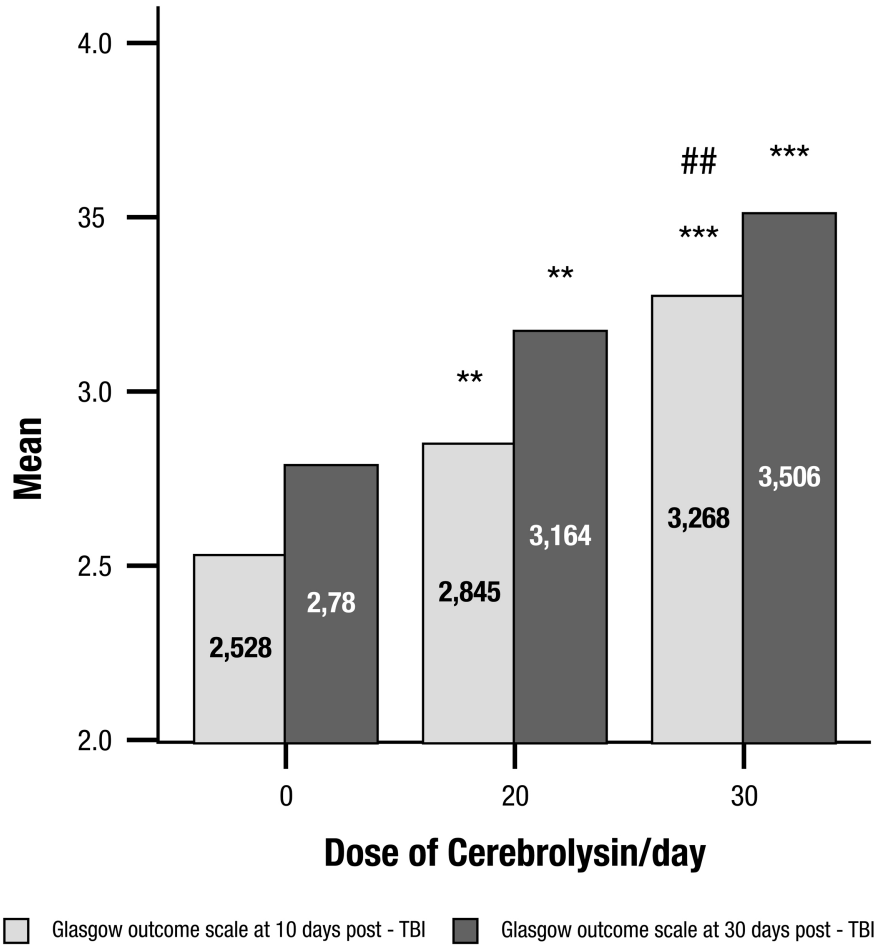

Fig. (5). GOS scores at 10 and 30 days post-TBI in the treatment groups of severe TBI patients. The control group is designated as Cerebrolysin 0 ml/day. At 10 and 30 days post-TBI, the average GOS score was significantly higher in both the 20 ml and 30 ml Cerebrolysin treatment groups compared with the control group. At 10 days, the average GOS score was significantly higher in the 30 ml Cerebrolysin treatment group compared with the 20 ml Cerebrolysin treatment group. ** = $p<0.01$ and *** = $p<0.001$ for the Bonferroni post-hoc test at 10 and 30 days compared with the controls; ## = $p < 0.01$ for Cerebrolysin 30 ml/day compared with Cerebrolysin 20 ml/day at 10 days.

DISCUSSION

TBI is a devastating medical condition that affects more than ten million people annually worldwide, constitutes the first cause of injury-related death in young adults, and is associated to high rates of lifelong impairments in physical, cognitive and psychosocial functioning [2]. The effective treatment of TBI patients should contribute to enhance not only survival but also the prevention of long-lasting disabilities by improving brain recovery after TBI. Although recent advances in the characterization of the cellular and molecular mechanisms involved in TBI pathophysiology allowed to identify novel therapeutic targets, almost all drug trials conducted to date failed to demonstrate clinical efficacy (see references 2 and 25 for review). Therefore, the development of an effective drug therapy for TBI represents an unmet need.

The results of this retrospective study indicate that add-on treatment with Cerebrolysin at doses of 20 mg/day and 30 mg/day improved the clinical recovery of TBI patients evaluated at 10 and 30 days after TBI compared with standard medical care alone. Cerebrolysin-induced improvements in the GOS and RDS measures of clinical outcome were statistically significant in the subgroup of mild patients at 10 days post-TBI only (Figs. **1**, **2**) and in the subgroups of moderate and severe TBI patients at both 10 days and 30 days post-TBI (Figs. **3**, **6**). These results are in agreement with the faster clinical recovery previously reported for moderate to severe TBI patients treated with Cerebrolysin in a double-blind controlled clinical trial [19] and are also supported by experimental studies [13-15] and other clinical trials [2, 16-18, 20, 21] showing the beneficial effects of Cerebrolysin on TBI outcome. Additionally, the safety and tolerability of Cerebrolysin in TBI patients was very good at doses of 20-30 ml/day.

Analyses of efficacy were performed separately for the subgroups of mild, moderate and severe TBI patients because there were relatively many more patients with moderate and severe TBI in the Cerebrolysin group than in the control group. Consistent with these group differences in the severity-related distribution of TBI patients, there were also differences in the average GCS scores at admission and in the percentages of patients presenting a comatose state, signs of intracranial hypertension or abnormal findings in

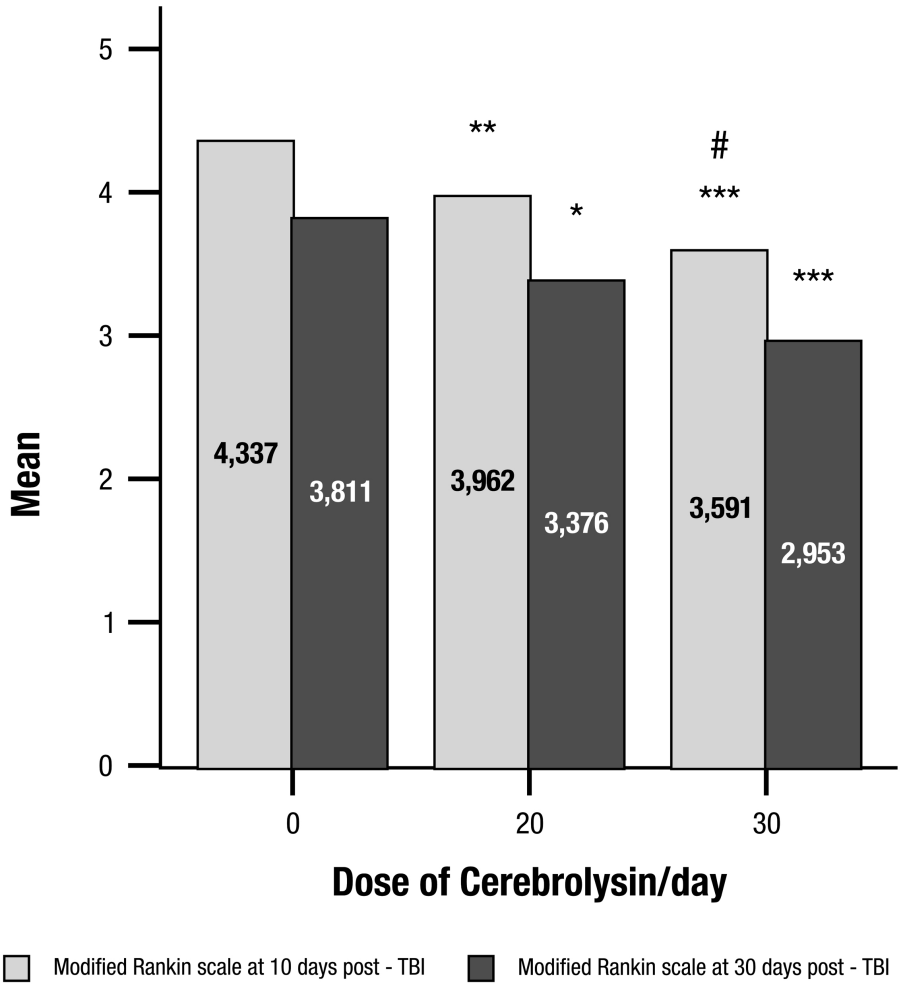

Fig. (6). RDS scores at 10 and 30 days post-TBI in the treatment groups of severe TIBI patients. The control group is designated as Cerebrolysin 0 ml/day. At 10 and 30 days post-TBI, the average RDS score was significantly lower in both the 20 ml and 30 ml Cerebrolysin treatment groups compared with the control group. At 10 days, the average RDS score was significantly lower in the 30 ml Cerebrolysin treatment group compared with the 20 ml Cerebrolysin treatment group. * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$ for the Bonferroni post-hoc test at 10 and 30 days compared with the controls; # = $p<0.05$ for Cerebrolysin 30 ml/day compared with Cerebrolysin 20 ml/day at 10 days.

cranial CT scan. Demographic and etiological characteristics of the patients were also different in Cerebrolysin and control groups (Table **1**). The clinical severity was analyzed in each subgroup to exclude potential bias resulting from better outcomes due to better initial clinical condition. In the mild TBI subgroup, the patients treated with both doses of Cerebrolysin had significantly lower average GCS scores (i.e., greater severity) at admission than the control patients. No significant differences in clinical severity were observed between the controls and Cerebrolysin-treated patients in the subgroup of moderate TBI. However, severe TBI patients treated with 30 ml/day Cerebrolysin had less severity (significantly higher mean GCS scores) at admission than the other severe TBI treatment groups (i.e., the control and 20 ml/day Cerebrolysin groups).

Our results revealed that mild TBI patients treated with Cerebrolysin (20 ml/day or 30 ml/day) had better outcomes than the controls at 10 days post-TBI but not at 30 days post-TBI. The outcome of mild-TBI patients at 30 days was excellent without specific treatment, which suggests that a 'ceiling effect' may explain the apparent lack of Cerebrolysin efficacy at this time point. In any case, the present findings are consistent with the results of previous studies showing a faster recovery of motor, cognitive and global clinical functioning during the first weeks after TBI, as well as an earlier hospital discharge of patients treated with Cerebrolysin [2, 18-21]. The positive effects of Cerebrolysin on clinical recovery observed in mild TBI patients are also supported by results of a recent placebo-controlled trial demonstrating that 30 ml Cerebrolysin improved the recovery of cognitive functions at 4 and 12 weeks after mild TBI [18].

However, moderate and severe TBI patients treated with Cerebrolysin (20 ml or 30 ml/day) had significantly better outcome scores than controls at both 10 days and 30 days post-TBI. These results are in line with findings of most of the previous clinical studies conducted with Cerebrolysin in TBI and showing positive treatment effects on acute recovery [2, 19], clinical outcome [2, 17, 20] and cognitive functioning [2, 16, 17, 19] in moderate to severe TBI

Table 5. Adverse events reported more frequently in each treatment group.

Adverse Event	Control N (%)	Cerebrolysin-20 N (%)	Cerebrolysin-30 N (%)	Significance (Chi-Square)
Nausea	291 (4.73)	37 (3.23)	21 (4.41)	X^2: 5.00; df:2; ns
Diarrhea	46 (0.74)	18 (1.57)	8 (1.68)	X^2: 10.34; df:2; p<0.01
Constipation	682 (11.08)	105 (9.19)	41 (8.61)	X^2: 5.85; df:2; ns
Urinary tract infection	249 (4.04)	61 (5.34)	15 (3.15)	X^2: 5.37; df:2; ns
Allergic reaction	21 (0.34)	3 (0.26)	1 (0.21)	X^2: 0.38; df:2; ns
Hypertension	348 (5.65)	72 (6.30)	19 (3.99)	X^2: 3.63; df:2; ns
Pyrexia	521 (8.47)	94 (8.23)	45 (9.45)	X^2: 0.67; df:2; ns
Insomnia	301 (4.89)	65 (5.69)	28 (5.88)	X^2: 1.97; df:2; ns

Differences between groups for the distribution of the indicated parameters were analyzed by using the chi-square test. The percentages refer to each particular group of patients.

patients. The results of the present study also showed dose-dependent effects of Cerebrolysin on outcome measures at 10 days post-TBI, particularly in the severe TBI patients. This finding appears to indicate that higher Cerebrolysin doses may result in a faster recovery in moderate and severe TBI patients, at least in the 20-30-ml dose range. Moreover, a longer duration of the Cerebrolysin treatment was found to be associated with better outcome scores in moderate and severe TBI patients at both 10 and 30 days post-TBI, which also indicates that Cerebrolysin improves TBI recovery in a dose-related manner. There have been no previous studies comparing the effects of different doses of Cerebrolysin on TBI outcome; thus, further controlled clinical trials are needed to confirm these preliminary dose-finding observations and to determine the most effective dose and treatment duration.

Although the specific molecular mechanisms underlying the positive actions of Cerebrolysin on TBI outcome were not investigated in the present study, according to results of experimental and clinical studies Cerebrolysin could improve short- and long-term recovery after TBI by interfering with several processes of secondary brain damage relevant for TBI pathology, including (2, 25): excitotoxicity associated to excessive glutamate release and calpain activation; oxidative stress induced by an increased generation of reactive oxygen species; sustained neuroinflammation with micoglia activation and enhanced levels of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-1beta; apoptosis and delayed neural damage involving caspase activation; diminished endogenous neurotrophic activity linked to reduced expression of brain-derived neurotrophic factor and to low circulating levels of insulin-like growth factor; misfolding and/or abnormal accumulation of beta-amyloid, tau and neurofilament proteins; and reduced neuroplasticity.

The effects of Cerebrolysin treatment on early TBI improvement may be explained by its neuroprotective actions on different pathogenic targets. The pleiotropic capacity of Cerebrolysin may be particularly important during acute neuroprotection, acting similar to trophic factors to modulate a variety of pathological mechanisms (i.e., excitotoxicity, inflammation, apoptosis, oxidative damage, *etc.*). Cerebrolysin may protect against oxidative and excitotoxic damage occurring after TBI at least in part by modulating inflammation and synaptic transmission [26, 27] and by reducing lipid peroxidation, the degradation of cytoskeletal proteins and calpain activation [6, 9, 28, 29]. The antioxidant activity of Cerebrolysin is supported by findings that its administration reduced brain levels of lipid peroxidation products in hypoglycemic mice [28], and significantly reduced blood levels of malondialdehyde (a reactive oxygen species generated by lipid peroxidation and biomarker of oxidative stress) in severe acute TBI patients [30]. In experimental conditions Cerebrolysin has been shown to inhibit the activity of the calcium-dependent proteases μ-calpain and m-calpain (6), whose overactivation may contribute to cytoskeletal and neuronal cell damage after TBI; and was able to rescue neurons from excitotoxic cell death induced by glutamate [31, 32], an excitatory amino acid showing an excessive release in TBI brains that seems to play a central role in the cascade of reactive events leading to secondary brain damage [2, 33]. Although the mechanism by which Cerebrolysin may counteract glutamate-induced neuronal cell damage in TBI is unknown, the recent finding that Cerebrolysin improves cognitive performance and enhances the hippocampal levels of glutamate in streptozotocin-diabetic rats suggests a modulatory rather than an inhibitory action of Cerebrolysin on glutamate transmission. An indirect mechanism through which Cerebrolysin might also contribute to reduce excitotoxic damage in TBI is *via* its modulatory effects on neuroinflammation. Elevations in inflammatory mediators such as TNF-α may produce excitotoxic and apoptotic damage after TBI by altering the expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ionotropic glutamate receptors and by enhancing caspase-dependent apoptosis [2, 34]. Cerebrolysin could counteract these mechanisms of injury by inducing a reduction of the elevated TNF-α levels in TBI patients similar to that demonstrated in diabetic rats and in Alzheimer's patients [33, 35].

Additionally, Cerebrolysin exerts neurotrophic effects by mimicking the activity of endogenous neurotrophic factors [2, 7, 8]. The compound supports neuronal cell survival, stimulates neuronal cell differentiation, growth and sprouting, and promotes the formation of synaptic contacts in cell cultures and in animal models [2, 7-11, 13-15]. Furthermore, Cerebrolysin enhances neurogenesis in different experimental conditions including closed head

injury [11, 13, 36, 37], which demonstrates that Cerebrolysin stimulates the restorative capacity of the brain after injury. This observation is important, as the upregulation of neurogenesis after TBI and ischemic stroke occurs endogenously and plays an important role in recovering neurological function [38]. Therefore, early and sustained Cerebrolysin treatment in TBI could provide clinical benefits by limiting cell death during the acute phase and by stimulating neuroplasticity and neurogenesis during the later recovery phase.

The occurrence of adverse events was similar, or even lower, in patients treated with Cerebrolysin and in those receiving standard medical care alone (Table **5**), except for diarrhea that was reported with a twofold higher frequency in the subgroups of Cerebrolysin-treated patients than in the control group. It is particularly important to note that there were no specific events of intolerance to Cerebrolysin like allergic or anaphylactic reactions. The good safety profile of Cerebrolysin demonstrated in this retrospective study is in line with the good tolerance previously reported and indicates that there are no concerns for the safe use of Cerebrolysin at doses of 20-30 ml/day in patients with mild, moderate or severe TBI.

Compared to previous drug trials, the present study includes one of the largest samples of TBI patients ever evaluated, approaching the size of the CRASH trial [25], is by far the biggest investigation with Cerebrolysin in TBI [2], and constitutes the only study involving mild, moderate and severe TBI patients for the evaluation of the severity-related efficacy of a drug. Our investigation also represents a real-world observational study, which provides information on the use of Cerebrolysin in TBI in the everyday medical practice. Based on the proportion of patients treated with Cerebrolysin in each severity-related group, it appears that Cerebrolysin is employed more frequently in moderate (40%) and severe (38%) TBI cases than in patients with mild injury (13.5%). And even in the mild TBI group, Cerebrolysin tended to be administered to the most severely affected cases as indicated by the lower GCS scores at admission in Cerebrolysin-treated cases compared to the control group. Among patients treated with Cerebrolysin, the dose of 20 ml/day was used in 83%, 77% and 46% of the mild, moderate and severe TBI cases, respectively; and the dose of 30 ml/day was predominantly employed in severe TBI (54% of the treated cases).

The main limitations of the present investigation are those of a retrospective study, the lack of randomization and placebo-control. Although selection criteria were established a posteriori, the lack of prospective inclusion criteria is another limitation of our study because decisions on the administration of the drug and the dose to be used were not controlled but relied on the experience and clinical judgment of each team. The imbalanced use of the two doses of Cerebrolysin, particularly in patients with mild and moderate TBI, reflects the general criteria of use in the real-world medical practice and represents another potential limitation. The severity-related analysis of the data helped to reduce differences among control and Cerebrolysin groups, but not completely. Other limitations of this study include the lack of evaluations on short- and long-term cognitive performance and survival rate, as well as on clinical outcome beyond one month. Results of the retrospective study reported here are also dimmed by differences in the clinical characteristics of the treatment groups. Future studies with Cerebrolysin such as the planed CAPTAIN trial are expected to benefit from the experience of the present investigation and to overcome its limitations by using a randomized-controlled design and evaluations of several functional domains during both the acute and post-acute TBI phases.

According to the results of this large retrospective study, Cerebrolysin seems to be effective in improving clinical outcome and functional recovery after traumatic brain injury. Cerebrolysin, acting as a neurotrophic multimodal agent with pleiotropic neuroprotective effects, may interfere with the pathogenic mechanisms of TBI at multiple levels to promote neuroprotection and neurorestoration [5]. The extrapolation of these positive results, however, is limited by the lack of randomization and control of such a retrospective study. Further prospective controlled clinical trials are needed to confirm the clinical efficacy of Cerebrolysin in TBI patients suggested by our results.

LIST OF ABBREVIATIONS

CT	=	Computerized Tomography
EDA	=	Endogenous Defense Activity
GCS	=	Glasgow Coma Scale
GOS	=	Glasgow Outcome Scale
RDS	=	Modified Rankin Disability Score
TBI	=	Traumatic Brain Injury
TNF-α	=	Tumor Necrosis Factor-Alpha

CONFLICT OF INTEREST

D.F. Muresanu was principal investigator in several clinical trials with Cerebrolysin and is member of the CAPTAIN trial scientific advisory board. No honorarium was received for this work.

A Alvarez was principal investigator in clinical trials and research projects with Cerebrolysin and is a member of EVER scientific advisory board for the EVE-AT-0412 trial. No honorarium was received to write this manuscript.

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D.F. Muresanu was the principle investigator of this study and contributed to its design and to writing the manuscript. The authors would like to thank G. Onose and C. Daia Chendreanu, for their support and assistance with this complex study.

REFERENCES

[1] Loane DJ, Faden AI. Neuroprotection for traumatic brain injury: translational challenges and emerging therapeutic strategies. Trends Pharmacol Sci 2010; 31: 596-604.

[2] Alvarez XA, Figueroa J, Muresanu D. Peptidergic drugs for the treatment of traumatic brain injury. Fut Neurol 2013; 8: 175-92.

[3] Mureşanu DF. Brain recovery—towards an unified theory. J Cell Mol Med 2010; 14: 455-6.

[4] Popescu BO. Still debating a cause and diagnostic criteria for Alzheimer's disease. J Cell Mol Med 2007; 11: 1225-6.

[5] Muresanu DF. Neurotrophic factor treatment of neurological disorders: the benefits of a pleiotropic treatment approach. CNS Neurol Disord Drug Targets 2011; 10: 415-6.

[6] Wronski R, Tompa P, Hutter-Paier B, Crailsheim K, Friedrich P, Windisch M. Inhibitory effect of a brain derived peptide preparation on the calcium dependent protease, calpain. J Neural Transm 2000; 107: 145-57.

[7] Akai F, Hiruma S, Sato T, *et al.* Neurotrophic factor-like effect of EPF1070 on septal cholinergic neurons after transections of fimbria-fornix in the rat brain. Histol Histopathol 1992; 7: 213-21.

[8] Satou T, Itoh T, Tamai Y, Ohde H, Anderson AJ, Hashimoto S. Neurotrophic effects of FPF-1070 (Cerebrolysin) on cultured neurons from chicken embryo dorsal root ganglia, ciliary ganglia, and sympathetic trunks. J Neural Transm 2000; 107: 1253-62.

[9] Schwab M, Antonow-Schlorke I, Zwiener U, Bauer R. Brain-derived peptides reduce the size of cerebral infarction and loss of MAP2 immunoreactivity after focal ischemia in rats. J Neural Transm Suppl 1998; 53: 299-311.

[10] Ren J, Sietsma D, Qiu S, Moessler H, Finklestein SP. Cerebrolysin enhances functional recovery following focal cerebral infarction in rats. Restor Neurol Neurosci 2007; 25: 25-31.

[11] Zhang C, Chopp M, Cui Y, *et al*. Cerebrolysin enhances neurogenesis in the ischemic brain and improves functional outcome after stroke. J Neurosci Res 2010; 88: 3275-81.

[12] Labiche LA, Grotta JC. Clinical trials for cytoprotection in stroke. NeuroRx 2004; 1: 46-70.

[13] Zhang Y, Chopp M, Meng Y, *et al*. Improvement in functional recovery with administration of Cerebrolysin after experimental closed head injury. J Neurosurg 2013; 118: 1343-55.

[14] Sharma HS, Zimmermann-Meinzingen S, Johanson CE. Cerebrolysin reduces blood-cerebrospinal fluid barrier permeability change, brain pathology, and functional deficits following traumatic brain injury in the rat. Ann NY Acad Sci 2010; 1199: 125-37.

[15] Valousková V, Francis-Turner L. Can Cerebrolysin influence chronic deterioration of spatial learning and memory? J Neural Transm Suppl 1998; 53: 343-9.

[16] Alvarez XA, Sampedro C, Figueroa J, *et al*. Reduction in qEEG slowing over and after treatment with Cerebrolysin in patients with moderate-sever traumatic brain injury. J Neural Transm 2008; 115: 683-92.

[17] Alvarez AX, Sampedro C, Perez P, *et al.* Positive effects of Cerebrolysin on electroencephalogram slowing, cognition and clinical outcome in patients with postacute traumatic brain injury: an exploratory study. Int Clin Psychopharmacol 2003; 18: 271-8.

[18] Chen CC, Wei ST, Tsaia SC, Chen XX, Cho DY. Cerebrolysin enhances cognitive recovery of mild traumatic brain injury patients: double-blind, placebo-controlled, randomized study. Br J Neurosurg 2013; 27: 803-7.

[19] König P, Waanders R, Witzmann A, *et al*. Cerebrolysin in traumatic brain injury – a pilot study of a neurotrophic and neurogenic agent in the treatment of acute traumatic brain injury. J Neurol Neurochir Psychiatr 2006; 7: 12-20.

[20] Onose G, Mureşanu DF, Ciurea AV, *et al*. Neuroprotective and consequent neurorehabilitative clinical outcomes, in patients treated with the pleiotropic drug cerebrolysin. J Med Life 2009; 2: 350-60.

[21] Wong GK, Zhu XL, Poon WS. Beneficial effect of cerebrolysin on moderate and severe head injury patients: result of a cohort study. Acta Neurochir Suppl 2005; 95: 59-60.

[22] Bullock R, Chesnut R, Ghajar J, *et al*. Guidelines for the management of severe traumatic brain injury. J Neurotrauma 2000; 17: 449-54.

[23] Bullock R, Chesnut R, Ghajar J, *et al*. Guidelines for the surgical management of traumatic brain injury. Neurosurgery 2006; 58: S2-62.

[24] Brain Trauma Foundation; American Association of Neurological Surgeons; Congress of Neurological Surgeons. Guidelines for the management of severe traumatic brain injury. J Neurotrauma 2007; 24 Suppl 1: S1-106.

[25] Beauchamp K, Mutlak H, Smith WR, Shohami E, Stahel PF. Pharmacology of traumatic brain injury: where is the "golden bullet"? Mol Med 2008; 14: 731-40.

[26] Alvarez XA, Lombardi VR, Fernández-Novoa L, *et al*. Cerebrolysin reduces microglial activation *in vivo* and *in vitro*: a potential mechanism of neuroprotection. J Neural Transm Suppl 2000; 59: 281-92.

[27] Xiong H, Baskys A, Wojtowicz JM. Brain-derived peptides inhibit synaptic transmission *via* presynaptic GABAB receptors in CA1 area of rat hippocampal slices. Brain Res 1996; 737: 188-94.

[28] Patocková J, Krsiak M, Marhol P, Tůmová E. Cerebrolysin inhibits lipid peroxidation induced by insulin hypoglycemia in the brain and heart of mice. Physiol Res 2003; 52: 455-60.

[29] Wronski R, Kronawetter S, Hutter-Paier B, Crailsheim K, Windisch M. A brain derived peptide preparation reduces the translation dependent loss of a cytoskeletal protein in primary cultured chicken neurons. J Neural Transm Suppl 2000; 59: 263-72.

[30] Fei Z, Shenyui YI. Changes of free radicals in the plasma of patients with craniocerebral trauma and therapeutic effect of Cerebrolysin. Chinese J Nerv Mental Dis 1992; 18: 6.

[31] Hutter-Paier B, Grygar E, Windisch M. Death of cultured telencephalon neurons induced by glutamate is reduced by the peptide derivative Cerebrolysin. J Neural Transm Suppl 1996; 47: 267-73.

[32] Riley C, Hutter-Paier B, Windisch M, Doppler E, Moessler H, Wronski R. A peptide preparation protects cells in organotypic brain slices against cell death after glutamate intoxication. J Neural Transm 2006: 113(1): 103-10.

[33] Georgy GS, Nassar NN, Mansour HA, Abdallah DM. Cerebrolysin Ameloriates Cognitive Deficits in Type III Diabetic Rats. PLoS One 2013; 8(6): e64847.

[34] Beattie MS, Ferguson AR, Bresnahan JC. AMPA-receptor trafficking and injury-induced cell death. Eur J Neurosci 2010; 32(2): 290-7.

[35] Alvarez XA, Sampedro C, Cacabelos R, et al. Reduced TNF-alpha and increased IGF-I levels in the serum of Alzheimer patients treated with the neurotrophic agent Cerebrolysin. Int J Neuropsychopharmacol 2009; 12: 867-72.

[36] Tatebayashi Y, Lee MH, Li L, Iqbal K, Grundkle-Iqbal I. The dentate gyrus neurogenesis: a therapeutic target for Alzheimer's disease. Acta Neuropathol 2003; 105: 225-32.

[37] Chen H, Tung YC, Li B, Iqbal K, Grundke-Iqbal I. Trophic factors counteract elevated FGF-2-induced inhibition of adult neurogenesis. Neurobiol Aging 2007; 28: 1148-62.

[38] Darsalia V, Heldmann U, Lindvall O, Kokaia Z. Stroke-induced neurogenesis in aged brain. Stroke 2005; 36: 1790-5.

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