NEURAL REGENERATION RESEARCH
Volume 8, Issue 5, February 2013

www.nrronline.org

doi:10.3969/j.issn.1673-5374.2013.05.010 [http://www.nrronline.org; http://www.sjzsyj.org]
Wu XN, Li ZS, Liu XY, Peng HY, Huang YJ, Luo GQ, Peng KR. Major ozonated autohemotherapy promotes the recovery of upper limb motor function in patients with acute cerebral infarction. Neural Regen Res. 2013;8(5):461-468.

Major ozonated autohemotherapy promotes the recovery of upper limb motor function in patients with acute cerebral infarction★

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Abstract

Major ozonated autohemotherapy is classically used in treating ischemic disorder of the lower limbs. In the present study, we performed major ozonated autohemotherapy treatment in patients with acute cerebral infarction, and assessed outcomes according to the U.S. National Institutes of Health Stroke Score, Modified Rankin Scale, and transcranial magnetic stimulation motor-evoked potential. Compared with the control group, the clinical total effective rate and the cortical potential rise rate of the upper limbs were significantly higher, the central motor conduction time of upper limb was significantly shorter, and the upper limb motor-evoked potential amplitude was significantly increased, in the ozone group. In the ozone group, the National Institutes of Health Stroke Score was positively correlated with the central motor conduction time and the motor-evoked potential amplitude of the upper limb. Central motor conduction time and motor-evoked potential amplitude of the upper limb may be effective indicators of motor-evoked potentials to assess upper limb motor function in cerebral infarct patients. Furthermore, major ozonated autohemotherapy may promote motor function recovery of the upper limb in patients with acute cerebral infarction.

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Received: 2012-11-08
Accepted: 2013-01-17
(N20120502001/WJ)

Key Words

neural regeneration; clinical practice; ozone; cerebral infarction; evoked potential; motor; upper limbs; upper limb paralysis; motor function; central motor conduction time; amplitude; National Institutes of Health Stroke Score; grants-supported paper; photographs-containing paper; neuroregenertion

Research Highlights

(1) Major ozonated autohemotherapy was evaluated by motor-evoked potential examination and clinical effect assessment.
(2) Major ozonated autohemotherapy promoted the recovery of upper limb motor function in patients with acute cerebral infarction.
(3) Effective indices of motor-evoked potential were used to assess central motor conduction time and amplitude of upper limb in cerebral infarction patients, and we found nerve conduction and recovery of motor function of the upper limb.

INTRODUCTION

Major ozonated autohemotherapy is a non-conventional therapy for ischemic disorders, particularly of the lower limbs[1-2]. Chronic limb ischemia is often accompanied by type 2 diabetes, and treatment with ozonated autohemotherapy can improve outcomes in these patients[3-4]. Although the

majority of studies using major ozonated autohemotherapy have treated chronic limb ischemia[5-8], there is some evidence of improved wound healing and limb salvage in patients with critical limb ischemia[9]. However, the mechanisms underlying the effects of major ozonated autohemotherapy for treatment of acute cerebral infarction remain unclear.

Previous studies have shown that ozone can improve outcomes from tissue ischemia, as follows. (1) Oxidation and oxygen saturation form the basis for the biological effects of ozone and provide energy for the ischemic organism. These actions of oxygen are critical for normal physiological functions in the human body. The oxidation reaction of glucose with oxygen provides the necessary energy to the body, and the complex from oxygen saturation is a vital component of the body. (2) A direct effect on partial brain tissue and cells: Ozone therapy can raise the saturation of blood oxygen, improve blood circulation, activate the metabolism of erythrocytes[10], and modify the degree of cerebral infarction ischemia. Ozone can also increase the content of adenosine triphosphate and 2,3-diphosphoglycerate in erythrocytes, causing a right shift of the oxygen dissociation curve, and promote blood and oxygen supply. Ozone activates pentose phosphate pathway carbohydrate metabolism pathways to increase the production of nicotinamide adenine dinucleotide phosphate, which is important for the maintenance of erythrocyte membrane integrity[11]. It was previously reported that ozone therapy caused a decrease in blood viscosity of peripheral anticoagulated whole blood, with the red color becoming darker, resulting in improved microcirculation[12] and enhanced tissue activity to improve oxygen supply and renew cell function, thus increasing the efficiency of anoxic metabolism. (3) Ozone can change the polymerization of blood plateletsto improve blood clotting in patients with cerebral infarction[12]. The production of hydrogen dioxide resolves the thrombus and decreases whole blood viscosity[11]. Ozone and hydrogen dioxide also accelerate the circulation of tricarboxylic acid, increase basal metabolism, and promote catabolism of fat to remove fatty materials, including color spots attached to the vessel wall. (4) Ozone can improve blood circulation and blood flow patterns in arteries and veins to increase the flexibility of erythrocytes[10], which increases blood flow through the capillaries and increases tissue oxygen supply. (5) Ozone provides ATP to hypoxic-ischemic brain tissue. For cells with an active metabolism acting *via* the pentose phosphate pathway, pentose phosphate pathway mainly provides the energy in the form of nicotinamide adenine dinucleotide phosphate of the monooxygenase system. Nicotinamide adenine dinucleotide phosphate is the coenzyme for glutathione reductase, and is critical for the maintenance of erythrocyte membrane integrity. After ozone therapy, the ATP content in erythrocytes also increases, indicating improved erythrocyte metabolism. Major ozonated autohemotherapy can maintain ATP and energy metabolism in brain tissues under conditions of ischemia and hypoxia, resulting in a decrease in cellular apoptosis[13]. (6) Ozone activates antioxidase and eliminates free radicals. It is well established that the mechanism of cerebral ischemia, especially cerebral edema and tissue damage caused by cerebral ischemia-reperfusion, is closely related to mass production of oxygen free radicals[14-15]. Chen *et al* [16] reported that the protective effect of ozone was strongly associated with nitric oxide production following increased expression of endothelial nitric oxide synthase and inducible nitric oxide synthase. The protective effect of ozone was also suggested to involve upregulation of the antioxidant defense system and beneficial effects on blood circulation and oxygen metabolism[17]. Finally, ozone preconditioning has a protective effect against skeletal bone ischemia/ reperfusion injury in rats by decreasing levels of malondialdehyde and protein carbonyl, and increasing activities of superoxide dismutase and glutathione peroxidase[18].

In a clinical study, Bocci *et al* [19] showed that major ozonated autohemotherapy treatment reduced chronic oxidative stress, delayed serious complications, and improved the quality of life of diabetic patients. Clavo *et al* [20] also reported preliminary Doppler findings of improved outcomes in patients with peripheral ischemic syndromes following ozone therapy. Ozone can rapidly combine with hemoglobin in the bloodstream to improve oxygen saturation, activate erythrocyte metabolism, and improve oxygen supply to brain tissue, resulting in improved blood circulation to the brain and improved brain cell activity; *i.e*., improves the ischemic penumbra zone hypoxia to promote the formation of collateral circulation and neural functional recovery[21]. Ozone preconditioning may have important clinical implications for protecting ischemia/reperfusion injury[22]. Nevertheless, previous studies have only used relatively small sample sizes. The motor-evoked potential of transcranial magnetic stimulation is an effective method for quantitative assessment of motor function in stroke patients. In this technique, magnetic stimulation is used to detect cortical motor-evoked potential and to determine the association of stroke with the damaged area and the absence of clinical

movement[23]. In 1993, Heald *et al* [24] discovered that patients with normal or extended central motor conduction time within 12–72 hours after the onset of stroke had a high survival rate with good functional recovery, while patients without motor-evoked potential recovered poorly. Ferbert *et al* [25] also reported that the degree of paralysis in stroke patients was directly proportional to motor-evoked potential amplitude.

Therefore, the aim of this clinical study was to determine the association between major ozonated autohemotherapy and functional recovery of patients with acute cerebral infarction using the National Institutes of Health Stroke Scale, the Modified Rankin Scale, and motor-evoked potential.

RESULTS

Quantitative analysis of subjects

A total of 86 acute cerebral infarction patients were equally divided into the ozone group and the control group. All patients received conventional therapy. Patients in the ozone group received major ozonated autohemotherapy.

During treatment, there was one case of cerebral hemorrhage in the control group, one case of upper gastrointestinal bleeding in each group, and one case of increased cerebral infarction area due to cerebral hernia in the ozone group. The clinical score was given ahead of schedule, while major ozonated autohemotherapy was terminated, and the motor-evoked potential examination was not reviewed. The clinical efficacy score of the patients with cerebral hemorrhage and brain herniation was not included in the comparison of the National Institutes of Health Stroke Scale and the Modified Rankin Scale.

Baseline data of subjects

There were a total of 86 acute cerebral infarction patients (58 males, 28 females). Paired *t*-test revealed no significant differences between the groups in terms of age, gender, height, blood glucose, low-density lipoprotein choresterol or interval time between onset and treatment ($P > 0.05$; Table 1).

Clinical efficacy of subjects

Before treatment, there was no significant difference in the National Institutes of Health Stroke Scale and the Modified Rankin Scale between the ozone and control groups ($P > 0.05$). However, the scores for the National Institutes of Health Stroke Scale and indices of the Modified Rankin Scale were significantly reduced after treatment for 10 ± 3 days ($P < 0.05$); the reduction in the control group was significantly less than that in the ozone group ($P < 0.05$; Table 2). The clinical total effective rate in the ozone group was significantly higher than that in the control group ($P < 0.05$; Table 3).

Table 1 Baseline data of the ozone and control groups

Item	Ozone group	Control group
Sex (male/female, *n*)	30/13	28/15
Age (mean ± SD, year)	64.13±11.21	63.85±10.87
Height (m)	1.68±0.06	1.69±0.07
Low-density lipoprotein cholesterol (mean ± SD, mM)	2.98±0.96	2.88±0.99
Blood glucose (mean ± SD, mM)	6.17±2.94	5.95± 2.43
Interval time between onset and treatment (mean ± SD, hour)	44.08±18.93	43.82±18.36

There were 43 patients in each group.

Table 2 National Institutes of Health Stroke Scale score and Modified Rankin Scale index in the ozone and control groups before and after treatment

Group	National Institutes of Health Stroke Scale score	
	Before treatment	After treatment
Control	11.5±3.8	7.3±4.1[a]
Ozone	11.7±3.5	5.4±3.5[ab]

Group	Modified Rankin Scale index	
	Before treatment	After treatment
Control	4.1±0.9	3.1±1.3[a]
Ozone	4.1±0.8	2.5±1.1[ab]

Results are expressed as mean ± SD, $n = 43$. [a]$P < 0.05$, *vs*. before treatment (paired *t*-test). [b]$P < 0.05$, *vs*. control group (multivariate analysis of variance).

Table 3 Comparison of efficacies between the ozone and control groups after treatment

Efficacy	Ozone group		Control group		*u*	*P*
	n	RANK	*n*	RANK		
Worse	2	1	2	1		
No effect	3	25.5	5	42.5		
Some improvement	12	348	21	609		
Significant improvement	24	1 548	14	903	2.07	< 0.05
Basic cure	2	170	1	85		
Total	43	2 092.5	43	1 640.5		

Basic cure, significant improvement, and some improvement were considered as effective outcomes of therapy. The clinical total effective rate in the ozone group was significantly increased compared with the control group ($P < 0.05$; rank sum test).

Motor-evoked potential results of subjects

Next, we analyzed the rise in cortical motor-evoked potential amplitude and the central motor conduction

time of the two groups before and after treatment (control group, *n* = 20; ozone group, *n* = 18).

Comparison of cortical potential rise rate before and after treatment

There was no significant difference in the cortical potential rise rate in 86 patients with acute cerebral infarction before treatment ($P > 0.05$). After ozone treatment for 10 ± 3 days, the number of cases of increased motor-evoked potential on the upper and lower limbs showed a non-significant increase ($P > 0.05$; Table 4).

Table 4 The change in the motor evoked potential rate [*n* (%)] of patients with acute cerebral infarction before and after treatment

Group		Before treatment	After treatment
Control	Upper limbs	21 (51.2)	28 (68.3)
	Lower limbs	31 (75.6)	36 (87.8)
Ozone	Upper limbs	20 (48.8)[a]	29 (70.7)[a]
	Lower limbs	29 (70.7)[b]	36 (87.8)[b]

The increase in the cortical potential rate was calculated as the number of cortical potential rise patients / total number × 100%. [a]$P < 0.05$, *vs*. the cortical potential rise rate of the upper limbs in the control group; [b]$P > 0.05$, *vs*. the cortical potential rise rate of the lower limbs in the control group; *n* = 41, binomial distribution.

Comparison of central motor conduction time before and after treatment

After treatment, central motor conduction time of the upper limb in the ozone group was significantly shorter than that in the control group ($P < 0.05$). There was no difference in the lower limb central motor conduction time between the two groups ($P > 0.05$). After treatment, the central motor conduction times of the upper and lower limbs in both groups were significantly shorter than those before treatment ($P < 0.05$; Figure 1).

Comparison of motor-evoked potential amplitude before and after treatment

After treatment, the cortical motor-evoked potential amplitudes of the upper limbs were significantly higher in the ozone group than in the control group ($P < 0.05$), while there was no difference in cortical motor- evoked potential amplitudes of the lower limbs between the groups ($P > 0.05$). The cortical motor-evoked potential amplitudes of the upper and lower limbs were significantly greater after treatment than those before treatment in both groups ($P < 0.05$; Figure 2).

Correlation analysis of National Institutes of Health Stroke Scale score to central motor conduction time and motor-evoked potential amplitude

The National Institutes of Health Stroke Scale improvement rate was positively correlated with the central motor conduction time improvement rate of the upper limb before and after treatment ($r = 0.78$) ($P < 0.05$), as well with the improvement rate of motor-evoked potential amplitude of the upper limb ($r = 0.85$, $P < 0.05$). However, there was no correlation with the central motor conduction time and motor-evoked potential amplitude of the lower limb ($P > 0.05$; Figure 3).

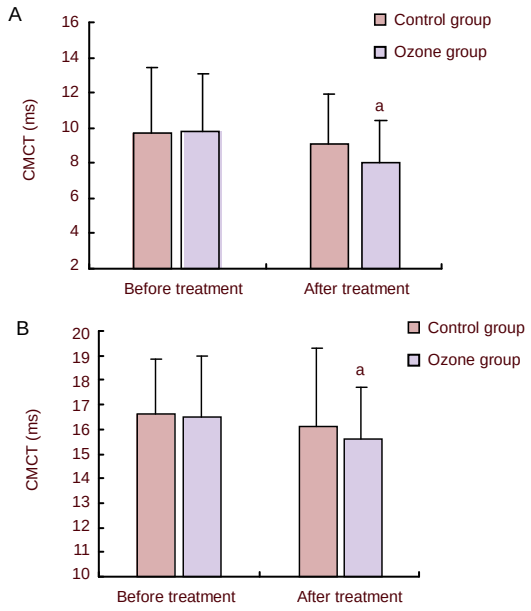

Figure 1 Central motor conduction time (CMCT) of upper (A) and lower (B) limbs in acute cerebral infarction patients before and after treatment.

(A) [a]$P < 0.05$, *vs*. control group (*n* = 18) for the CMCT of the upper limb (*n* = 20). (B) [a]$P > 0.05$, *vs*. control group (*n* = 18) for the CMCT of the lower limb (*n* = 20; multivariate analysis of variance).

DISCUSSION

Ozone is an oxygen isomeride made up of three atoms of oxygen, and is a strong oxidant with two free electrons. In 2 000, ozone was approved in Europe for clinical treatment of virus hepatitis. Since then, ozone has been more widely applied in the treatment of systemic diseases and cerebral infarction. Nevertheless, there are only a few clinical studies of ozone therapy for treatment of acute cerebral infarction. Clavo *et al* [20] assessed the effect of ozone therapy on Doppler blood flow in the middle cerebral and common carotid arteries, and reported an improvement in peripheral ischemic syndromes.

The potential for ozone therapy as a complementary treatment in cerebral low perfusion syndromes merits further clinical evaluation. However, the underlying mechanisms that result in successful treatment are not well known.

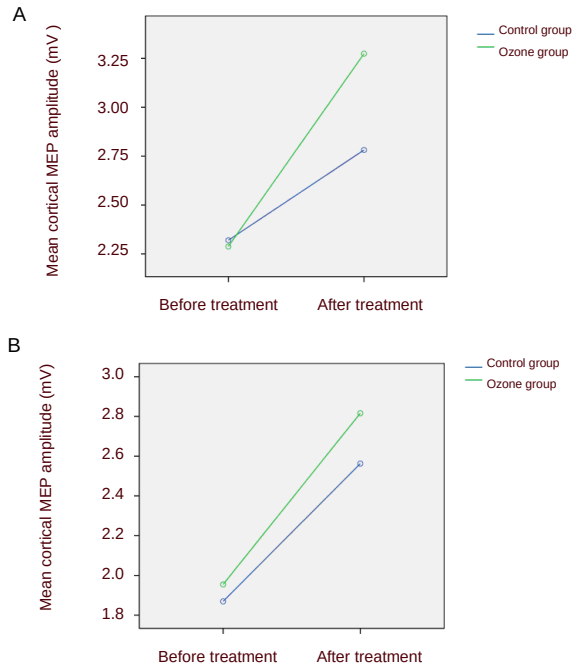

Figure 2 Comparison of cortical motor-evoked potential (MEP) amplitude of the upper (A) and lower (B) limbs in acute cerebral infarction patients before and after treatment.

(A) $P < 0.05$ for the comparison of cortical MEP amplitude of the upper limb in ozone group ($n = 18$) and control group ($n = 20$). (B) $P > 0.05$ for the comparison of cortical MEP amplitude of the lower limb in the ozone group ($n = 18$) and the control group ($n = 20$; paired-sample Wilcoxon signed rank sum test).

In the present study, we assessed the effects of major ozonated autohemotherapy on motor function recovery of patients with acute cerebral infarction according to the U.S. National Institutes of Health Stroke score, Modified Rankin Scale score, and transcranial magnetic stimulation motor-evoked potential. We found that after major ozonated autohemotherapy, the National Institutes of Health Stroke Scale and Modified Rankin Scale scores of patients with acute cerebral infarction were decreased compared with the control group, while the total clinical effective rate was higher than the control group. Furthermore, after major ozonated autohemotherapy, the rate of increase in motor-evoked potential amplitude of the upper limbs in the affected side was significantly increased, while the central motor conduction time of the upper limb in the ozone group was significantly decreased, compared with those in the control group. These data suggest that major ozonated autohemotherapy can promote motor function recovery in patients with acute cerebral infarction.

In addition, using the relative analysis method, both preoperative and postoperative National Institutes of Health Stroke Scale scores were associated with central motor conduction time and motor-evoked potential amplitude of the upper limbs, while there was no relationship with central motor conduction time and motor-evoked potential amplitude of the lower limbs. These data suggest that central motor conduction time and motor-evoked potential amplitude of the upper limbs can directly respond to the motor functions of patients. Furthermore, motor-evoked potential is an efficient method for evaluating motor function of stroke patients.

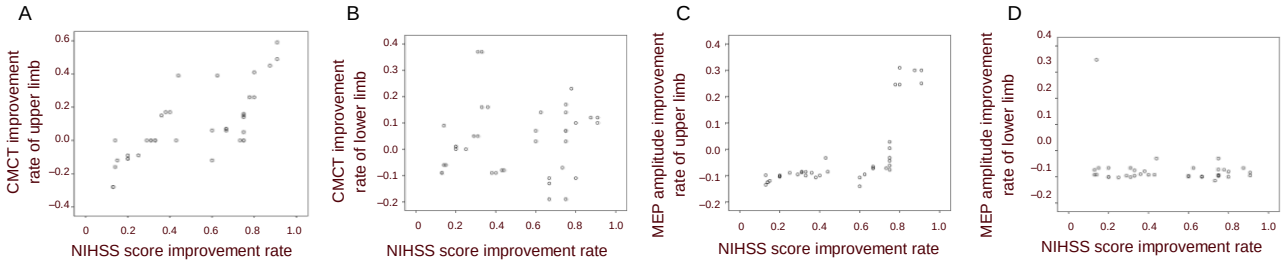

Figure 3 Analysis of correlation of the National Institutes of Health Stroke Scale (NIHSS) to central motor conduction time (CMCT) and motor-evoked potential (MEP) amplitude.

NIHSS score improvement rate was calculated as the (NIHSS scores before treatment – NIHSS scores after treatment)/NIHSS scores before treatment × 100%. The CMCT improvement rate was calculated as the (CMCT before treatment – CMCT after treatment)/CMCT before treatment × 100%. The MEP amplitude improvement rate was calculated as the (MEP amplitude before treatment – MEP amplitude after treatment)/MEP amplitude before treatment × 100%.

(A) Scatter graph of the relationship between NIHSS improvement rate and the CMCT improvement rate of the upper limbs. The NIHSS improvement rate was positively correlated with the CMCT improvement rate ($r = 0.78$, $P < 0.05$). (B) Scatter graph of the relationship between the NIHSS improvement rate and the CMCT improvement rate of the lower limbs. There was no correlation between these values ($P > 0.05$).

(C) Scatter graph of the relationship between the NIHSS improvement rate and the MEP amplitude improvement rate of the upper limbs. The NIHSS improvement rate was positively correlated with the MEP amplitude improvement rate ($r = 0.85$, $P < 0.05$). (D) Scatter graph of the relationship between the NIHSS improvement rate and the MEP amplitude improvement rate of the lower limbs. There was no correlation between these values ($P > 0.05$, $n = 38$). Spearman rank correlation analysis was performed.

Nevertheless, there are a number of disadvantages using motor-evoked potential examination, such as the technical limitations of inspectors and pain induced in the patients. These factors should be improved in future studies to allow faster examinations. Moreover, we only observed a temporary treatment effectiveness. Future studies are required with larger sample volumes and long-term follow-up in all patients to further evaluate the efficacy of major ozonated autohemotherapy. Finally, although we found a positive outcome for the use of major ozonated autohemotherapy as a complementary treatment in acute cerebral infarction, the underlying mechanisms that result in successful treatment remain unclear.

In conclusion, major ozonated autohemotherapy treatment during the early stage of acute cerebral infarction significantly increased the clinical National Institutes of Health Stroke Scale and the Modified Rankin Scale scores, shortened the central motor conduction time, and increased the motor-evoked potential amplitude of the upper limbs in motor-evoked potential, suggesting that major ozonated autohemotherapy can improve motor function. Thus, major ozonated autohemotherapy may be an efficient treatment for patients with acute cerebral infarction.

SUBJECTS AND METHODS

Design

A controlled, prospective study.

Time and setting

The study was performed at Guangzhou General Hospital of Guangzhou Military Command, China from May 2008 to November 2009.

Subjects

Diagnostic criteria of patients with cerebral infarction

Blood supply disturbances in the brain are induced by various causes and often result in brain ischemia or ischemic necrosis, which corresponds with neurological deficits over one to several days. The injury is often confined to the blood supply region of a certain artery. In the present study, cerebral infarction was confirmed by history and physical examination and brain MRI[27].

Inclusion criteria

(1) Patients were included from 30–80 years of age. (2) Acute cerebral infarction that was not induced by tumor, traumatic brain injury, brain parasitic diseases, or metabolic disorder was confirmed through CT or MRI. (3) The National Institutes of Health Stroke Scale score was between 6–21. (4) Diseased time of patients was more than 6 hours, but less than 72 hours, and patients included were those who could not be treated with intravenous thrombolysis.

Exclusion criteria

(1) Patients with severe stroke, such as multi-lobe infarction (low density in CT larger than 1/3 cerebral hemisphere). (2) Patients subjected to thrombolytic therapy. (3) Patients with shock, severe heart and lung complications and liver dysfunction, or life expectancy of less than 1 month. (4) Patients with coagulation dysfunction or abnormal amount of platelet, including thalassemia, sickle cell anemia, and glucose-6- phosphate dehydrogenase deficiency (favism). (5) Hyperthyroidism symptoms are not under control. (6) High-sensitivity and ozone allergy. (7) Using kinase or anti-free radical agents. (8) Pregnant or lactating women. (9) Not suitable for motor-evoked potential, including a pacemaker or with a history of epilepsy. The program was discussed and adopted by Ethics Committee of Guangzhou General Hospital of Guangzhou Military Command, China, and all subjects signed the informed consent.

In total, 86 patients were included in this study, which was performed in accordance with requirements of *Declaration of Helsinki* and *Administrative Regulations on Medical Institution*, formulated by the State Council of China[26]. Written informed consent was obtained from all patients.

Methods

Drug treatment and ozone therapy

The conventional standard treatment guidelines were based on the a guide to early treatment issued by the Stoke Affiliated Society of American Heart Association in 2003[27], and included: (1) stroke unit care; (2) monitoring and treatment of complications such as hypertension, arrhythmia, and high blood glucose; and (3) treatment of neurological complications. The basic medicines used by patients in both groups were 450 mg *Xueshuantong* injection (a traditional Chinese patent medicine; the main component is arasaponin; Lyophilized, Zhunzi Z20025652, Lot No. 11100407; Guangxi Wuzhou Pharmaceutical Co., Ltd., Wuzhou, Guangxi Zhuang Autonomous Region, China) and 0.1 g aspirin (Bayer HealthCare Manufacturing S.r.l, Beijing, China), or 75 mg Clopidogrel bisulfate tablet (Sanofi Winthrop Industries, France), once per day. In the ozone group, the patients were treated with major ozonated autohemotherapy, once per day, for 10 ± 3 days.

An ozone generating device (HUMARES, Bruchsal, Germany) was used for the major ozonated autohemotherapy. The operating procedure was as follows: 100 mL blood was collected from the cubital vein of patients into a sterile closed blood bag with 10 mL of 2.5% sodium citrate. The blood was then mixed with 100 mL ozone (47 μg/mL) for approximately 2 minutes, and then re-transfused fast through the initial intravenous access (< 30 minutes) into the patient.

Evaluation of treatment efficacy

Evaluation of clinical motor function

Neurological deficits before and after a cycle of 10 ± 3 days treatment were evaluated using the National Institutes of Health Stroke Scale and the Modified Rankin Scale score[28]. Basic cure: 91–100% reduction of impairment scores, disability level 0; significant improvement: 46–90% reduction of impairment score, disability level 1–3; some improvement: impairment score decreased by 18–45%; no effect: impairment score decreased by 17% or less; worse: impairment score increased more than 18%; death and the events causing test suspension were all classified as worse situation. The basic cure, significant improvement, and some improvement conditions were considered as effective outcomes.

Analysis of cortical motor function by cortical motor-evoked potential

Motor-evoked potential examination was applied using The KeypointR.net EMG evoked potential equipment (Danndy, Skovlunde, Denmark) and the MagPro magnetic stimulator (Danndy), with a circular 90 mm hand-held coin. The center of the coin was placed at the vertex or slightly lateral toward the stimulated hemisphere. Face 'A' (visible face) was used for left hemisphere stimulation and face 'B' for right hemisphere stimulation. Slight displacements were made in all directions until the position yielding the lowest threshold was found. Cortical functional areas and the C_7 nerve root/L_5–S_1 nerve root were stimulated by the MagPro magnetic stimulator when the patients were lying in the supine and sitting positions. The cortical stimulation intensity was 85–90% of the maximum output, and the nerve root stimulation intensity was 55%. The magnetic motor-evoked potential latency was defined as the shortest latency from eight responses. The surface electrodes were recorded at the abductor muscle of the thumb of the upper limb and the tibialis anterior muscle of the lower limb. The recording electrode (diameter 0.8 cm) was set at the muscle venter, the reference electrode (diameter 0.8 cm) was at the tendon, and the ground wire was fixed in the contralateral limb. For the patients whose muscle strength was too low to be stimulated, the record was taken through a slight voluntary contraction or a passive activity[29]. When the peripheral nerve root was stimulated, the motor-evoked potential with the longest incubation period was considered as the nerve root motor potential[5]. The shortest period from the beginning of cortical stimulation to production of muscle contraction was recorded as the total motor conduction time. The central motor conduction time was calculated by subtracting the nerve root motor potential from the total motor conduction time.

Statistical analysis

Normally distributed data were recorded as mean ± SD, and the M (QR) was used for recording skewed distribution data. SPSS 16.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis. Ranked data were tested by the rank sum test. Measurement data within the group were compared with a paired *t*-test. Percentages were compared using the binomial distribution. Multivariate analysis of variance was used to compare trends before and after treatment between the ozone and control groups, and the paired-sample Wilcoxon rank sum test was used to test the heterogeneity of variance. The Spearman rank correlation analysis was used for correlation analyses. A value of $P < 0.05$ was considered statistically significant.

Acknowledgments: We thank Dr. Gerd Wasser (German doctor, Vice Chairman of the European Associate of Ozone Therapy) for original ideas and useful theory, Professor Xiaofeng He (Nanfang Hospital in China, President of Chinese Federation of Ozone Therapy) for valuable guidance and extensive research experience on ozone therapy, and Miss Yifang Zhang for her English suggestions of this thesis.

Funding: This study was supported by the Guangdong Province Medical Science Research Fund, No. B200258.

Author contributions: Xiaona Wu provided, integrated, and analyzed data, wrote manuscript and obtained funding. Zhensheng Li analyzed data and contributed to statistical analysis. Xiaoyan Liu provided clinical data and contributed to motor-evoked potential examination. Haiyan Peng and Yongjun Huang contributed to motor-evoked potential examination. Gaoquan Luo provided clinical data. Kairun Peng conceived and designed the study, and revised the manuscript. All authors approved the final version of the paper.

Conflicts of interest: None declared.

Ethical approval: This study received permission from the Ethics Committee of Guangzhou General Hospital of Guangzhou Military Command, China.

Author statements: The manuscript is original, has not been submitted to and is not under consideration by another

publication, nor has it been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/patent application/funding source disputations.

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(Edited by Guo YB, He XF/Qiu Y/Song LP)