Reversal Of Acute Human Brain Ischemic Injury By Lysine Induced Therapeutic Angiogenesis: Preliminary Results Of A Pilot Study

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Citation

R Gupta, A Agarwal, S Agarwal, P Mohan, M Husain, J Alger, C Pandey, D Datta. *Reversal Of Acute Human Brain Ischemic Injury By Lysine Induced Therapeutic Angiogenesis: Preliminary Results Of A Pilot Study*. The Internet Journal of Neurology. 2004 Volume 4 Number 1.

Abstract

Effects of L-lysine monohydrochloride on ischemia reversibility were studied using diffusion and perfusion MRI in 11 patients and 4 controls with large territory ischemic brain injury. Parametric maps of vascular physiological function were synthesized. Region of interest analysis was done from the infracted and corresponding contralateral normal regions. Statistical analysis demonstrated significant changes in terms of lesion volumes, National Institute of Health Stroke Scale (NIHSS) scores, relative cerebral blood volume (rCBV), relative cerebral blood flow (rCBF), mean transit time (MTT) ratios in patients treated with lysine compared to controls. A significant increase in rCBV ratio in lysine treated patients suggests that increased angiogenesis is probably responsible for tissue reversibility and functional recovery in these patients.

INTRODUCTION

It is established that by 6 months after a stroke about 20-30% of patients have died, 20-30% are moderately to severely disabled, 20-25% have mild to moderate disability, and the remainder are without deficit [1, 2]. Initial clinical deficits can improve dramatically or worsen during the first 48-72 hrs [2,3,4]. Changes in the clinical status might sometimes be related to pathophysiologic events such as early reperfusion, hemorrhagic transformation or edema of the ischemic lesions [3]. Thrombolytic therapy using recombinant tissue plasminogen activator (rt-PA) has significantly improved the neurological outcome when administered up to 3 hours after the onset of symptoms [5,6,7,8]. However, the brief therapeutic window of 3 hours significantly limits the number of treatable patients. There are concerns about the relatively high rate of symptomatic intracranial hemorrhage in patients treated with intra-arterial thrombolysis [7]. Unfortunately, there has been a lack of progress in acute stroke drug development since the efficacy of rt-PA was demonstrated on the basis of National Institute of Neurological Disorders and Stroke trials in 1995 [7]. Other drugs intended to facilitate reperfusion, such as intra-arterial thrombolysis with prourokinase and the defibrinogenating agent ancord, given intravenously, have shown promise [7].

Despite numerous studies of neuroprotective compounds showing reduction of infarct volume in animal stroke models and in some cases, promising phase II results, none has been proven efficacious on the basis of a positive phase III trial. On this basis it is clear that novel approaches will need to be considered and employed in the future clinical trials [7].

Potential of diffusion and perfusion MRI in selecting patients for thrombolysis beyond 3 hours and evaluating the tissue effect of reperfusion has been realized [9]. Parsons et al suggested that patient selection by perfusion and diffusion MRI and its evaluation of response to treatment may identify the patients in whom intravenous rt-PA therapy will be of clinical benefit when therapy is initiated between 3 and 6 hours [10]. Hyperacute lesion volume of more than 89 ml imaged with diffusion weighting imaging (DWI) may be predictive of early neurological deterioration [9]. A combination of relative peak height and time to peak perfusion measurements may be a better predictor of infarct growth than quantitative haemodynamic parameters [9,11].

Angiogenesis is defined as the formation of new blood vessels by sprouting of endothelial cells from pre-existing vessels. During the process of sprouting, endothelial cells degrade the underlying basement membrane, migrate into

the neighboring tissue, proliferate and assemble into tubes. Finally, tube-to-tube connections are made and blood flow is established [12]. Among the factors capable of modulating angiogenesis characterized to date, vascular endothelium growth factor (VEGF) is the best candidate for a specific regulator of endothelial growth and differentiation. VEGF is expressed in the normal adult brain, mainly in the epithelial cells of choroids plexus, but also in astrocytes and neurons [12]. Recurrent hypoxic conditions are associated with a considerable increase in the cerebral microvasculature network in children perinatally [13]. Examination of autopsied brains of South American Indians living at high altitudes and permanently exposed to hypoxia has revealed an increase in microvascular density [14]. VEGF has been used to induce angiogenesis in a rat model of focal cerebral embolic ischemia and can markedly enhance angiogenesis in the ischemic brain and reduces neurological deficits during stroke recovery [15].

A large number of molecules are being tested for promotion of therapeutic angiogenesis including VEGF. L- lysine monohydrochloride (L-lysine) has been shown to promote therapeutic angiogenesis in wound healing [16,17]. We present our preliminary pilot data in patients with large territory ischemic brain injury where we have analyzed the effects of this molecule on ischemia reversibility for the first time in literature.

MATERIALS AND METHODS

ELIGIBILITY AND INCLUSION CRITERIA

Patients with symptoms of acute hemispheric stroke presenting to either Neurology Department of KGMU or to the Neuroscience Department of Mayo Medical Center Lucknow were taken up for the imaging study at MR Section Department of Radiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India. The ethical clearance was taken from Institute's Ethical Committee for performing the human study. The inclusion criteria for the L-lysine study were the presence of main territory or its major branch infarction, restricted diffusion as evidenced by the apparent diffusion coefficient (ADC) values of less than 0.6 x 10^{-3} mm^2 /sec and consent for the patient for willingness to participate in the study. Presence of large hemorrhage as evidenced by MRI, patients with diabetes mellitus and with history of previous stroke or preexisting neurological or psychiatric illness were excluded from the study. All patients presented within a mean duration of 29.16 hrs with range between 22-58 hrs except for one patient who presented on the 14 th day and was included on the basis of low ADC.

A total of 15 patients fulfilling the above criteria were taken up for the study. Four patients that declined to be treated with L-lysine were followed up using the same protocol as used for lysine treated patient. There were 13 males and 2 females with age ranging between 40 to 75 years. L-lysine was administered intravenously in the dose of 60-mg/kg body weight in four divided doses for 7 days immediately after the imaging. This dose calculation was based on the use of oral lysine in brain and myocardial ischemia previously [18].

Clinical outcome was assessed by using the National Institute of Health Stroke Scale [(NIHSS); baseline, 7 th day, and 6 weeks] and modified Rankin scale [(mRS); 90 th day] by which outcome was classified in terms of independence (mRS score 0, 1, 2) or severe disability or death (mRS score 3 through 6)

MRI METHODS

MR imaging was performed on a 1.5-T whole-body imager (Signa; General Electric Medical Systems, Milwaukee, WI, USA) equipped with echo-planar imaging data acquisition capability. Baseline MRI scans included axial T2-weighted, T1-weighted, fluid attenuated inversion recovery (FLAIR), DWI, perfusion weighted imaging (PWI), and intracranial magnetic resonance angiography (MRA) sequences.

Follow-up MRI scans were obtained at 7 th day (T2, T1, FLAIR, DWI, PWI, MRA) and at 6 weeks (T2, T1, and MRA). MR angiograms in all the three studies were performed to demonstrate the site of vascular occlusion and to look for any change occurring during a period of 6 weeks in terms of re-canalization. DWIs were acquired by using 5 mm slice thickness and no inter slice gap, field-of-view (FOV) of 240 mm. Two levels of diffusion sensitization (b-values= 0 and 1,000 sec/mm^2), the later applied in each of three principal gradient directions (x, y, and z) were used to calculate the ADC. Perfusion MRI was performed by using the bolus passage of contrast method (Gadolinium-Diethylene triaminepentaacetic acid; 0.1 mmol/kg dose via hand injection) with spin echo echo-planar imaging. Thirty five single shot echo planar images were obtained in each of 11 slices in 67 sec. Imaging parameters were TR/TE=1900/85, FOV of 30X30 cm, image matrix of 128X128 pixels, slice thickness of 10 mm with no inter slice gap. Parametric maps of vascular physiological function were synthesized by using an arterial input deconvolution

paradigm obtained from UCLA stroke group [9, 11].

DATA ANALYSIS

The relative cerebral blood volume (rCBV), relative cerebral blood flow (rCBF) and mean transit time (MTT) maps were generated and region of interest (ROI) analysis was done from the infracted region and corresponding contralateral normal region. The values of these parameters were expressed as the ratio of abnormal/normal region [19] for initial and follow-up perfusion study and will be used as rCBV, rCBF and MTT ratios in the remainder of the manuscript.

Two investigators blinded to the clinical data using an open consensus method without initial independent ratings performed MRI volume measures jointly (MA, PM). DWI and T2 lesion volumes were first identified by visual inspection for regions of abnormality, and then measured by outlining regions of interest by hand. Day 7 infarct volumes were calculated by using the larger lesion from the DWI/T2 sequence. Final volume was calculated at 6 weeks on the basis of T2-weighted images.

Using SPSS software statistical analysis was performed. Changes in the lesion volumes, NIHSS scores, rCBV, rCBF, MTT ratios in patients with (lysine treated group) and without lysine (non-lysine treated group) were analyzed by using the Wilcoxon signed Rank Test. p value less than 0.05 was considered significant. Since it was a pilot study, subjects were not randomized.

RESULTS

The results of this study are summarized in table 1. Most of these patients presented to us between 22-58 hours after the onset of the event except for a patient of complete MCA occlusion who was recruited on the 14 th day on the basis of low ADC on imaging. MR angiogram revealed occlusion of the main vessel or its major branch in 14 of the 15 cases and occlusion was persistent even at 6 weeks of stroke at the same position (figures 1-3). There were seven patients with complete middle cerebral artery (MCA) or its major branch occlusion, three with internal carotid artery (ICA), two with posterior cerebral artery (PCA), and one each with basilar and vertebral artery occlusion. One patient had embolic stroke with normal intracranial MR angiogram.

Figure 1

Table 1: Summary of clinical and imaging data.

Parameters	N	Before Lysine			After Lysine*			Wilcoxon Signed Ranks Test (p)
Group –I (On Lysine)		Mean	±SD	Median	Mean	±SD	Median	
NIHSS	11	11.36	3.50	11.00	5.55	3.86	5.00	0.003
DWI/T2 Volume	11	35.69	33.88	31.30	23.22	22.24	17.95	0.004
rCBF ratio	11	0.35	0.25	0.39	0.98	0.48	0.88	0.003
rCBV ratio	11	0.47	0.25	0.46	1.05	0.32	1.05	0.003
MTT ratio	11	3.20	0.93	2.76	1.26	0.95	0.94	0.003
Group-II (No Lysine)*								
NIHSS	4	15.67	5.77	19.00	12.33	8.96	17.00	0.11
DWI/T2 Volume (cc)	4	61.33	44.21	41.90	41.76	25.52	49.65	0.29
rCBF ratio	4	0.42	0.21	0.30	0.43	0.60	.012	1.00
rCBV ratio	4	0.71	0.06	0.69	0.61	0.42	0.39	1.00
MTT ratio	4	3.48	1.02	3.98	3.06	2.04	4.12	1.00

NIHSS= National Institute of Health Stroke Scale, DWI= diffusion weighed imaging, rCBF= relative cerebral blood flow, rCBV= relative cerebral blood volume, MTT= mean transit time, *= in-group II with no lysine this data represent with out lysine after one week, cc=cubic centimeter

The results of the study are summarized in table 1. In lysine treated group, the Mean NIHSS score was 11.36 ± 3.5 that changed to 5.55 ± 3.86 after one week and 2.36 ± 3.67 after 6 weeks of follow up. In patients who were followed up without lysine administration had initial NIHSS 15.67 ± 5.77 that changed to 12.33 ± 8.96 after one week and 8.0 ± 7.0 after 6 weeks. 10 out of 11 patients (90.9%) on lysine treatment showed mRS between 0 to 2 at the end of three months and one patient showed disability of more than 3. Of the 4 patients who were not on lysine treatment, the mRS scale was more than 3 in three patients and less than three in one patient (25%). The mean infracted tissue volume measured 35.69± 33.88 cc on the initial study that changed to 23.22 ± 22.24 cc after one week of lysine (n=11) and this decrease in volume was statistically significant (p=0.004) (figure 1). However, in the non-lysine group (n=4), the volume did not decrease significantly (p= 0.29) (figure 2). rCBF ratio, rCBV ratio and MTT ratio showed significant changes before and after one week of lysine treatment in these values (figure 1). The MTT ratio showed significant decrease (p=0.003) from 3.20 ± 0.93 to 1.26 ± 0.95 while rCBV and rCBF ratios showed significant increase (p=0.003 and 0.003) from 0.47 ± 0.25 and 0.35 ± 0.25 to 1.05 ± 0.32 and 0.98 ± 0.48 respectively from pre lysine to one-week post lysine treatment.

Figure 2

Figure 1: MR images of lysine treated patient presented with left hemiplegia and aphasia and right middle cerebral artery (MCA) territory infarction presented after 22 hours of event at baseline (I), on 7 th day of treatment (II), and at 6 th week (III). MR images from left-to- right are arranged as T2-weighted, T1-weighted, apparent diffusion coefficient (ADC), diffusion weighted imaging (DWI) (b=0 and 1000), and perfusion weighted imaging (PWI) [relative cerebral blood flow (rCBF), relative cerebral blood volume (rCBV), and mean transit time (MTT) ratios]. At baseline (I), T2-weighted axial MR image showing infarct appearing slightly hyperintense, which on T1-weighted image appear as isointense. The infarct shows restricted diffusion on DWI with low ADC value. Follow up after one week of lysine (II) shows decrease in MTT ratio and increase in rCBF and rCBV ratios compared to the first study. 2 nd follow up at 6 th week (III) demonstrates increased T1 hyperintensity consistent with methemoglobin. MR angiogram shows occlusion of right internal carotid artery (ICA) even at 6 th week as seen in the first study. National Institute of Health Stoke Scale (NIHSS) score changed from 18 at baseline (I) to 3 on 7 th day (II) and finally to 0 at 6 th week. Modified Rankin Sclae (mRS) after 3 months was 0.

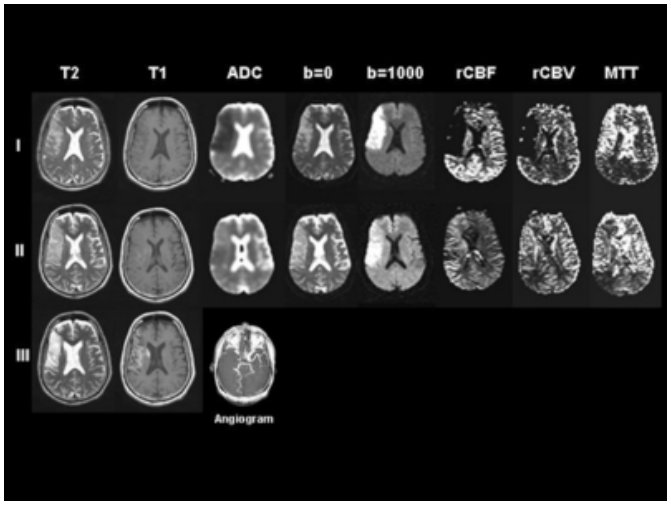


Figure 3

Figure 2: MR images of lysine treated patient with hemiparesis and instability along with right vertebral artery (VA) occlusion presented after 28 hours of event at baseline (I), at 7 th day of treatment (II), and at 6 th week (III). MR images from left-to- right are arranged as T2-, T1-, ADC, DWI (b=0 and 1000), and PWI (rCBF, rCBV, and MTT). T2-weighted MR image at baseline (I) shows bilateral cerebellar infarct and restricted diffusion on DWI. Follow up MRI after one week of lysine shows reduction in the infarct volume on DWI. PWI images similar to the first case show decrease in MTT ratio and increase in rCBF and rCBV ratios compared to the first study. 2 nd follow up at 6 th week illustrates increased T1-hyperintensity consistent with methemoglobin. MR angiogram in the III study shows occlusion of right VA as was present in the first study. NIHSS score changed from 7 at baseline to 2 on 7 th day of treatment and subsequently to 0 at 6 th week. The mRS after 3 months was 0.

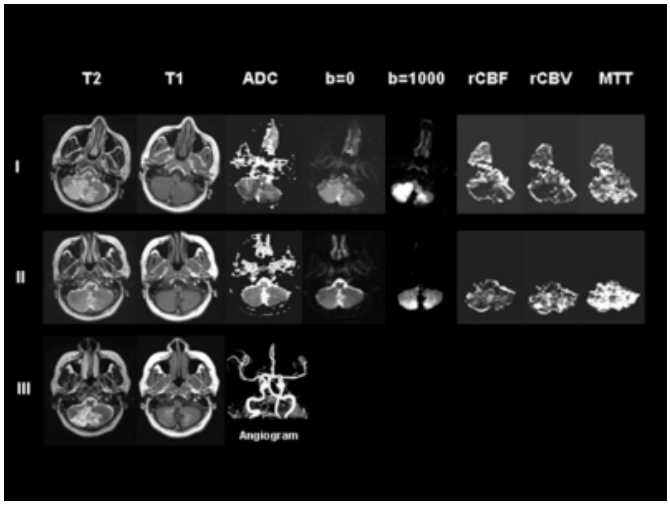
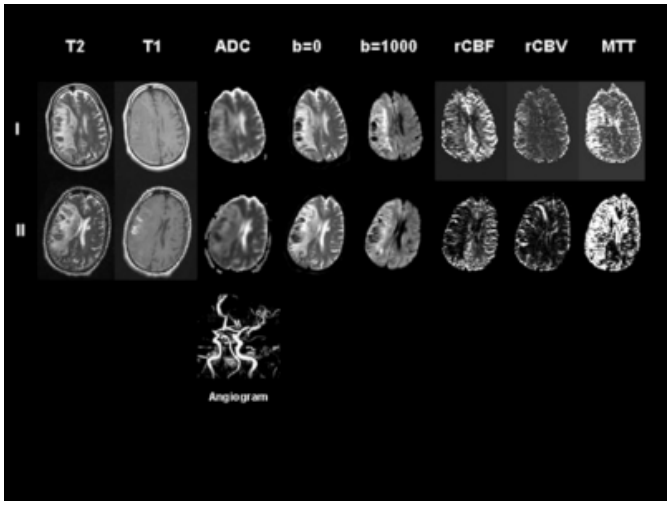


Figure 4

Figure 3: MR images of patient not treated with lysine, with left hemiplegia and right MCA occlusion presented after 48 hours of event at baseline (I), on 7 th day (II), and at 6 th week (III). MR images from left-to-right are arranged as T2-weighted, T1-weighted, ADC, DWI (b=0 and 1000), and PWI (rCBF, rCBV, and MTT). First study at baseline shows larger infarct area on the right cerebral hemispehere. Follow up on 7 th day showed relative increase in infarct volume and decrease in rCBF and rCBV ratios and increase in MTT ratio compared to first study (I). MR angiogram at 6 th week shows right MCA occlusion similar to the first study. NIHSS score at baseline was 18 that changed to 17 on 7 th day and 14 at 6 th week. The mRS was recorded as 4 at 3 months.



There was no correlation between the infarct volume with NIHSS scale either in the lysine treated patients or in the non–lysine treated patients. In all the patients who were treated with lysine, there was a relatively increased presence of T1 hyperintensity, consistent with methemoglobin, in the region of the residual infarct involving the cortical gray matter or periventricular region in all the patients where lysine treatment was given compared to the ones where no lysine was injected.

DISCUSSION

Our preliminary pilot data suggests significant functional and image morphological recovery in patients with lysine group compared to non-lysine group. The recovery on the basis of modified Rankin scale of disability showed no disability or minimal independent disability in 10/11 patients compared to non-lysine group that showed no disability in one out of four patients at three months. Greshem et al have analyzed the residual disability in survivors of stroke patients and found that only 20-25% of the patients remained without deficit [2]. Our data is consistent with the literature that only one patient (25%) had no residual disability at 3 months and remaining had moderate to severe disability who were followed up with conventional treatment without lysine. The recovery with no or minimal disability in 10/11 patients clearly suggests that lysine is responsible for restitution of functionality in these patients.

DWI may delineate infracted brain tissue within minutes, although there is a growing evidence that in the very early stage of stroke there may be reversible DWI changes in up to 45% of patients experiencing recanalization after treatment with rtPA [5,6, 20,21,22]. However, these lesion reversals in most instances are only minor or partial and quite frequently not permanent and there are contradictory data as to whether a DWI/ADC threshold for reversible ischemia exists [22]. The current study is based on the presumption that reduction in ADC represents cytotoxic edema or a combination of cytotoxic and vasogenic edema. The infracted tissue with restricted ADC may not represent tissue necrosis and may still show some reversibility. We have shown that stroke volume reduced significantly after a week of lysine administration with no significant change at 6 weeks suggests that there is partial reversibility of ischemic tissue after one week of lysine administration. Thrombolysis has been mainly used in patients with MCA occlusion; however some recent studies are available where it has been tried in posterior circulation stroke with some success [5]. In the present study, anterior as well as posterior circulation stroke showed similarity of functional recovery.

PWI in stroke defines the area of cerebral hypoperfusion. The absolute volume difference or ratio of PWI and DWI reveals the ischemic tissue at risk of irreversible infarction [11, 18]. In a 60 minutes transient focal cerebral ischemia model, rCBV has been shown to increase significantly on day 7 and then decreased on the day 14 [23]. Wu et al assessed rCBV in 11 patients with stroke varying from 20 hours to 25 months and found a significantly lower value in infracted region compared to contralateral normal region [24]. They also noted a much lower rCBV in the core of the infarct compared to periphery; however the overall rCBV was significantly lower than the contralateral normal region. It has been found that rCBV has been shown to correlate with microvascular density that may be the result of recruitment of new collaterals and angiogenesis [25]. Since, rCBV is known as an indicator of angiogenesis, we have used rCBV for in vivo quantification of angiogenesis with dynamic contrast MRI. In an animal study, it has been

shown that angiogenesis starts within 96 hours of occlusion of the MCA and reaches peak at 7 days [23]. Angiogenesis has been shown to correlate with improved survival rate on pathological study of the human brain that died after stroke at variable period [26]. In the present study there was a significant increase in rCBV and rCBF ratios and significant decrease in MTT ratio after 7 days in all patients who were on lysine compared to ones not on lysine treatment. Reversibility in cases of ischemic brain injury is achieved either by recanalization or by re-vascularization (angiogenesis) at the ischemic site. Usually patients with non-recanalization show poor recovery in natural course as well as the ones not able to get recanalized on intra-arterial and or intravenous thrombolytic agents [8]. In our study, none of our patients showed recanalization on MR angiogram up the 6 th week. All 11 patients treated with lysine showed marked recovery in terms of significant changes in rCBV, rCBF, and MTT ratio as compared to the 4 non-lysine treated patients. This observation suggests that functional recovery in these 11 patients is probably due to lysine-induced angiogenesis in the ischemic tissue and not because of recanalization.

L-lysine is widely available as a non-prescription oral supplement. Most of the pharmaceutical grade product is used as a suppressant of recurrent herpes simplex infections [19]. Recently, Yamori et al have done a controlled trial in stroke prone rats and found that 87% of controls had stroke at 25 weeks while animals on lysine supplementation had significantly lower incidence of stroke [27]. Datta et al demonstrated angiogenic capability of lysine in wound healing for the first time. They showed induction of profound angiogenic response on histopathology in cutaneous acute and chronic wounds on topical application of L-lysine [16, 17]. It supports maximum cellular growth and expansion in serum containing media than in a serum free media suggesting an action mediated through growth factors [16, 17]. Lysine mediated angiogenesis is postulated to be an end result of the molecule acting as cell surface bridge binding the angiogenic factors to their receptors. This helps in augmenting the angiogenesis induced by the lysine molecule due to ligand-receptor binding process [16]. The angiogenic property of this molecule has been utilized in this study.

VEGF is an angiogenic growth factor that binds to two high affinity receptors and transient and permanent MCA occlusion in rats evokes its expression in the ischaemic brain [15] suggesting that after a stroke, VEGF may be involved in angiogenesis. Zhang et al demonstrated a significantly enhanced cerebral microvascular perfusion and improved functional neurological recovery when rhVEGF165 was administered to ischemic rats at 48 hours [15]. Their data indicates that late treatment with rhVEGF165 enhances angiogenesis in ischemic brain. In the present study, the lysine is being used to efficiently utilize the available VEGF that is expressed by the ischemic vascular endothelium that has significantly improved the clinical recovery and perfusion parameter after one week. All these patients presented to us in this study after 22 hours and lysine effect of perfusion and clinical recovery could be seen after a week.

Thrombolysis therapy has been established as a treatment of choice between 3-6 hours after the onset of non-hemorrhagic stroke. However this initial pilot study suggests that recruitment of patients even after 24 hrs of stoke may help in widening the window with respect to the patient selection for the treatment. There are several limitations of this study. It is not a controlled open or double blind study and the sample size is small. So the rigorous statistical analysis was not possible. However, the initial data suggests significant functional and imaging recovery with non-recanalization of the occluded vessel. The perfusion changes in post lysine group including significant increase in rCBV ratio, a surrogate marker of angiogenesis suggest that increased angiogenesis is probably responsible for tissue reversibility and functional recovery in these patients with acute brain ischemic injury. Though the results of initial pilot data appear encouraging, a large systematic open label controlled trial is needed to reach a definite conclusion.

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References

1. Allen CMC. Predicting the outcome after a stroke: a prognostic score. J Neurol Neurosurg Psych 1984;47:475-480.
2. Greshem GE, Fitzpatrick TE, Wolf PA, McNamara PM, Kannel WB, Dawer TR. Residual disability in survivors of stroke-the Framingham Study. N Engl J Med 1975;293;954-956.
3. Baird AE, Austin MC, McKay WJ, Donnan GA. Changes in cerebral tissue perfusion during the first 48 hours of

ischaemic stroke: relation to clinical outcome. J Neurol Neurosurg Psych 1996;61:26-29.
4. Davalos A, Cendra E, Teruel J, Martinez M, Genis D. Deteriorating ischaemic stroke: risk factor and prognosis. Neurology 1990;40:1865-1869.
5. Schellinger PD, Fiebach JB, Hacke W. Imaging-based decision making in thrombolytic therapy for ischemic stroke present status. Stroke 2003;34:575-583.
6. Kidwell CS, Saver JL, Mattiello J, Starkman S, Jahan R, Vespa P, Kalafut M, Alger JR. Thrombolytic reversal of acute human cerebral ischemic injury shown by diffusion/perfusion magnetic resonance imaging. Ann Neurol 2000;47:462-469.
7. Wardlaw JM, Sandercock PAG, Berge E. Thrombolytic therapy with recombinant tissue plasminogen activator for acute ischemic stroke. Where do we go from here? A cumulative meta-analysis. Stroke 2003;34:1437-1442.
8. Rother J, Schellinger PD, Gass A, Siebler M, Villringer A, Fiebach J, Jansen O, Kucinski T, Schoder V, Szabo K, Junge-Hulsing GJ, Hennerici M, Zeumer H, Sartor K, Weiller C, Hacke W. Effect of intravenous thrombolysis on MRI parameters and functional outcome in acute stroke less than 6 hours. Stroke 2002;33:2438-2445.
9. Warach S. Stroke neuroimaging. Stroke 2003;34:345-347.
10. Parson MW, Barber PA, Chalk J, Darby DG, Ross S, Desmond PM, Gerraty RP, Tress BM, Wright PM, Donnan GA, Davis SM. Diffusion- and perfusion-weighted MRI response to thrombolysis in stroke. Ann Neurol 2002;51:28-37.
11. Shih LC, Saver JL, Alger JR, Starkman S, Leary MC, Vinuela F, Duckwiler G, Gobin P, Jahan R, Villablanca P, Vespa PM, Kidwell CS. Perfusion-weighted magnetic resonance imaging thresholds identifying core, irreversibly infracted tissue. Stroke 2003;34:1425-1430.
12. Marti HJH, Bernaudin M, Bellail A, Schoch H, Euler M, Edwige P, Risau W. Hypoxia-induced vascular endothelial growth factor expression precedes neovascularization after cerebral ischemia. Am J Pathol 2000;156:965-976.
13. Kaluza J, Adamek D, Mierzynski W. Morphological patterns of central nervous system changes in children with cyanotic and non-cynotic heart failure: morphometry of brain vasculature in congenital heart disease. Neuropathol Pol 1988;1:49-59.
14. Cerves-Navarro J, Gertz H, Frydl V. Cerebral blood vessel changes in old people. Mech Ageing Dev 1987;39:223-231.
15. Zhang ZG, Zhang L, Jiang Q, Zhang R, Davies K, Powers C, van Bruggen N, Chopp M. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. J Clin Invest 2000;106:829-838.
16. Datta D, Bhinge A, Chandran V. Lysine: is it worth more? Cytotechnology 2001;36:3-32.
17. Datta D. Essential amino acid lysine and its analogues support faster and qualitatively better healing of wounds. http://www.pharma-transfer.com, 2000.
18. Flodin NW. The metabolic roles, pharmacology, and toxicology of lysine. J Am Coll Nutrition 1997;16:7-21.
19. Schaefer PW, Ozsunar Y, He J, Hamberg LM, Hunter GJ, Sorensen AG, Koroshetz WJ, Gonzalez RG. Assessing tissue viability with MR diffusion and perfusion imaging. Am J Neuroradiol 2003;24:436-443.
20. Kidwell CS, Alger JR, Di Salle F, Starkman S, Villablanca P, Bentson J, Saver JL. Diffusion MRI in patients with transient ischemic attacks. Stroke 1999;30:1174- 1180.
21. Chalela JA, Ezzeddine MA, Calabrese TM, Latour LL, Baird AE, Luby ML, Warach S. Diffusion and perfusion changes two hours after intravenous rt-PA therapy: a preliminary report. Stroke 2002;33:356-357.
22. Schaefer PW, Hassankhani A, Christopher R, Koroshetz WJ, Rordorf G, Schwamm LH, Beonanno F, Gonzalez RG. Partial reversal of DWI abnormalities in stroke patients undergoing thrombolysis: evidence of DWI and ADC thresholds. Stroke 2002;33:357.
23. Lin T, Sun S, Cheung W, Li F, Chang C. Dynamic changes in cerebral blood flow and angiogenesis after focal cerebral ischemia in rats evaluation with serial magnetic resonance imaging. Stroke 2002;33:2985-2991.
24. Wu RH, Bruening R, Berchtenbreiter C, Weber J, Steiger HJ, Peller M, Penzkofer H, Reiser M. MRI assessment of cerebral blood volume in patients with brain infarcts. Neuroradiology 1998;40:496-502.
25. Roche MA, Dunn JF, Makki M, Abajian M, Daghlian CP, Springett R, Merlis J, Lu SY. Monitoring angiogenesis in normal brain using steady-state quantification of R2 (SSTAR2) with MION infusion. Proc Intl Soc Magn Reson Med 2003;11:1932.
26. Krupinski J, Kaluza J, Kumar P, Kumar S, Wang JM. Role of angiogenesis in patients with cerebral ischemic stroke. Stroke 1994;25:1794-1798.
27. Yamori Y. Hypertensive cerebrovascular diseases: importance of nutrition in pathogenesis an prevention. Ann NY Acad Sci 1993;676:92-104.

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