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Endogenous defense mechanism-based neuroprotection in large-vessel acute ischemic stroke: A hope for future

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Abstract:

BACKGROUND: Stroke is a leading cause of morbidity and mortality worldwide and a leading cause of disability. None of the neuroprotective agents have been approved internationally except edaravone in Japanese guidelines in acute ischemic stroke. We here discuss that there are two types of endogenous defense mechanisms (EDMs) after acute stroke for neuromodulation and neuroregeneration, and if both can be activated simultaneously, then we can have better recovery in stroke.

AIMS AND OBJECTIVES: We aimed to study the effect of combination of neuroprotection therapies acting on the two wings of EDM in acute large-vessel middle cerebral artery (LMCA) ischemic stroke.

METHODS: Sixty patients of LMCA stroke were enrolled and randomized within 72 h into two groups of 30 patients each. The control group received standard medical care without any neuroprotective agents while the intervention group received standard medical care combined with oral citicoline with vinpocetine for 3 months with initial 1 week intravenous and edaravone and cerebrolysin injection, started within 72 h of onset of stroke. Patients were assessed on the basis of the National Institutes of Health Stroke Scale, Fugl-Meyer Assessment Score, Glasgow Coma Scale, and Mini-Mental Status Examination at admission, discharge, and after 90 days.

RESULTS: The intervention group showed significant and early improvements in motor as well as cognitive recovery.

CONCLUSION: Combination therapy for neuroprotection which is acting on two pathways of EDM can be useful in functional recovery after acute ischemic stroke.

Keywords:

Endogenous stroke neuroprotection, multimodal neuroprotection therapy, vinpocetine in stroke

Introduction

Stroke-related disability is the most common cause of global disease burden.[1-3] Due to many reasons, stroke is now considered a chronic neurological condition rather than monophasic acute illness.[2] Even after new advancement in treatment of acute stroke, more than half of survivors had to live with at least one functional disability (motor, sensor, or cognitive) for the rest of their life.[2] One Indian study on stroke survivors showed the prevalence of persistent disability according to the severity, and it was mild in 42.4%, moderate in 43%, and severe in 14.6%, respectively.[4] Large-vessel occlusion by far is the most common and most disabling type of stroke as compared to small-vessel disease.[5]

Globally, efforts have been made to reduce deficits and improve neurological outcome after large-vessel stroke with addition of neuroprotective agents with usual medical treatment. In the last two decades, many pharmacological agents have been tried that can improve in better neurological outcome after stroke.[6,7] Few

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important agents which were tested as neuroprotective agents are selective serotonin reuptake inhibitors,[8-10] citicoline,[11,12] edaravone,[13,14] cerebrolysin,[15-17] and Phosphodiesterase Inhibitors (PDEIs)[18-20] Other newer neuroprotective therapies described in literature are growth factors, monoclonal antibodies, drugs, cell-based therapies, activity-based therapies, and brain stimulation-based therapies.[21] Unfortunately, after such extensive search, none of the neuroprotective agents had failed to get the approval by American, European, and Indian stroke guidelines.[1,22] Till date, edaravone is the only neuroprotective agent that could get approval by the international stroke guideline (Japanese Guidelines 2015, level B approval).[14] Failure and success of trials on neuroprotective agents in stroke might be multifactorial and had been highlighted by Cheng *et al*.[7]

After acute stroke, the cascade of primary and secondary neuronal injuries begins along with the activation of endogenous defense pathway which is responsible for minimizing the insult. The activation of endogenous defense mechanism (EDM) is responsible for recovery by neuronal protection, neuronal repair, and neuronal regeneration.[23-25] EDM itself consists of two important processes to activate the pathways of recovery in stroke, (1) "absolute pathway" and (2) "relative pathway."[25] The "absolute pathway" of EDM is based on gene expression and protein synthesis which helps for neuronal restoration by the synthesis and migration of neurotrophic factors or neurotrophic-like molecules. Another "relative pathway" of EDM is dependent of agonists/antagonists for various ion channels and receptors with anti-inflammatory, anti-oxidative, and anti-excitotoxin activity for neuronal protection.[25] Thus, it can be presumed that multimodal molecular mechanisms such as poor expression of neuromodulation protein synthesis with inflammation, oxidative stress, excitotoxicity, and mitochondrial failure which are finally responsible poor expression of neuronal plasticity signaling pathway and results in poor recovery of stroke.[23-25] It had also been postulated by researches that neuroprotection therapies with multimodal action can be the better option to reduce the stroke-related disability.[23-25]

Our hypothesis is that if we can simultaneously modulate both "absolute" and "relative" pathways of EDM, then we can get better results with neuroprotective agents after stroke. That was the basis of our current study in which we have used multimodal neuroprotection acting on both wings of EDM in large-vessel middle cerebral artery (LMCA) stroke and compared it with usual medical treatment without neuroprotective agents.

Methods

This intention-to-treat trial was aimed to find out the effectiveness of neuroprotective polytherapy in motor outcome after first acute LMCA territory cortical infarction. Patients with first attack of acute LMCA ischemic stroke with the National Institutes of Health Stroke Scale (NIHSS) score of ≥6 were enrolled, and random allocation in two groups was done with sealed envelope technique; Group 1: it was intervention group in which patients presenting with first attack of acute LMCA infarction within 72 h of onset and put on usual medical treatment for ischemic stroke along with neuroprotective polytherapy and Group 2: usual medical treatment for stroke without any neuroprotective agents; the study design was approved by the institutional ethical committee and written consent was obtained by the subject.

LMCA cortical infarction was defined when we had radiological evidence of acute cortical involvement of LMCA territory infarction either atherothrombotic or cardioembolic. LMCA stroke included both M1 and M2 syndromes on a clinical and radiological basis. Large-vessel occlusion (intracranial or extracranial) was diagnosed by carotid Doppler and computed tomography/magnetic resonance imaging angiography. We have selected patients having first ever stroke who presented to the hospital within 72 hours of onset of symptoms. This means that there was no clinical as well as radiological evidence of prior ischemic or hemorrhagic stroke. The time duration of 72 hours was calculated from the time last seen normal.

Exclusion criteria included those patients of LMCA stroke who did not give consent, had serious comorbid illness like kidney failure on hemodialysis, cancers, already chair/bed bound and having disabling neurodegenerative illness like dementia or Parkinson's disease, who had complete or partial reversal after thrombolysis, minor stroke (NIHSS ≤5), and small-vessel lacunar stroke.

The control group was given standard medical treatment according to Indian guidelines published in 2019. The components of standard medical care or treatment are based on the current guidelines of acute stroke management from the Ministry of Health and Welfare in 2019.

All patients with measurable neurological deficit after acute ischemic stroke who can be treated within 4.5 h after symptom onset should be evaluated without delay to determine their eligibility for treatment with a thrombolytic therapy (medical or surgical).

All acute stroke patients should be given at least 150 mg of plain aspirin immediately after excluding intracranial hemorrhage with neuroimaging (in patients receiving thrombolysis, aspirin should be delayed until after the 24 h postthrombolysis).

Patients with acute ischemic stroke who are allergic to or intolerant of aspirin should be given an alternative antiplatelet agent (e.g., clopidogrel).

In patients with large hemispheric infarct (malignant middle cerebral artery [MCA] territory infarct), aspirin may be delayed until surgery. Aspirin may be started after decision is made not to operate.

In dysphagic patients, aspirin may be given by enteral tube. 20. For noncardioembolic stroke, aspirin (at least 75 mg) should be continued as indicated in "secondary prevention." Any patient with acute ischemic stroke who is known to have dyspepsia with aspirin should be given a proton-pump inhibitor in addition to aspirin (also see secondary prevention).

Patients with indication for neurosurgery should be referred to a center with neurosurgical facility.

Along with specific treatment, general principles of management were followed in all patients including the ABCDEF Protocol which is: (a) airway management, (b) blood pressure management, (c) cardiac care, (d) decongesting therapy for brain edema and Deep Vein thrombosis (DVT) prevention, (e) epileptic seizure management, and (f) feeding for nutritional support.

All cases were subjected to early mobilization and rehab technique for neural recovery.

The intervention group was subjected to the following neuroprotective polytherapy protocol in addition to standard medical treatment: (a) acting on absolute pathway of EDM which included (1) injection cerebrolysin 30 mg twice daily for 7-day IV infusion and (2) vinpocetine 10 mg daily oral route for 12 weeks and (b) acting on relative pathway of EDM which included (1) injection edaravone 30 mg twice daily for 7-day IV infusion and (2) citicoline 500 twice daily for 12 weeks. All neuroprotective agents were started within 72 h of acute stroke. Patients were given the same medical treatment irrespective of thrombolytic therapy or hemorrhagic transformation of MCA stroke.

Severity of stroke

Severity of stroke was assessed at the time of admission with the Glasgow Coma Scale (GCS), NIHSS, and Mini-Mental Status Examination (MMSE).

Primary outcome measure

Primary outcome was measured by the Modified Rankin Scale (MRS), at discharge and 90 days, along with the Fugl-Meyer Scale (FMS) in upper and lower limbs at day 0, day of discharge, 30 days, and 90 days were documented to assess the motor outcome.

Secondary outcomes measures

GCS, NIHSS, and MMSE scores were assessed at discharge and at 90 days for assessment of stroke recovery. Patients, who have achieved the MRS score of 2 in follow-up, were asked about the time to achieve this unassisted previous daily activity.

Statistical analysis

IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp was used. Fisher's exact test, Chi-square test for group and Student's t-test for the mean were used for statistical analysis.

Results

Among 77 patients, who had new onset LMCA territorial stroke and presented within 72 h, 17 patients either died or lost in follow-up. The remaining 60 patients were randomly allocated to either control group or intervention group (30 in each). The mean age of the patients was 60.2 years (range: 31–84 years). The demography and profile of these patients are given in Table 1, which showed that both the groups were homogenous in baseline parameters. None of the patients had received thrombolytic therapy in our cohort.

Table 2 shows dynamic changes in stroke severity parameters over 90-day follow-up. At the time of admission, only 4 (13.3%) patients in the intervention group had GCS of more than 13, while at the end of

Table 1: General demographic parameters in two groups

Parameters	Group 1 (intervention) (*n*=30), *n* (%)	Group 2 (control) (*n*=30), *n* (%)	*P*
Gender (male)	20 (67)	21 (70)	0.5
Diabetic	13 (43.3)	14 (46.6)	0.5
Hypertensive	18 (60)	18 (60)	0.603
Active smokers	17 (56.6)	17 (56.6)	0.603
Daily alcohol user	7 (23.3)	8 (26.6)	0.5
Cardiac cause for stroke	6 (20)	7 (23.3)	0.5
Atrial fibrillation	6 (20)	4 (13.3)	0.365
Significant intracranial or extracranial large-vessel occlusion of >50%	19 (63.3)	17 (56.6)	0.396
LDL level >150 mg/dl	22 (73.3)	17 (56.6)	0.139
Hyperhomocysteinemia	8 (26.6)	8 (26.6)	0.614
Left middle cerebral artery stroke	17 (56.6)	18 (60)	0.5

LDL: Low-density lipoprotein

3 months, 73.3% had GCS of >13. The intervention group had significantly better GCS at 90 days (P = 0.032) as compared to the control group. At the end of 3 months, the intervention group had that a higher proportion of patients had <6 NIHSS which was not statistically significant (30% vs. 13.3%, P = 0.33). Significantly, a high proportion of patients had MRS of 0–2 (minimal disability) in the intervention group at 90 days (P = 0.000).

Table 3 shows the changes in motor, cognitive, and overall disability-related outcomes at the end of 3 months.

Table 2: Change in severity parameters over 90 days in two groups

Parameters	Group 1 (intervention) (*n*=30), *n* (%)	Group 2 (control) (*n*=30), *n* (%)	*P*
GCS of >13 at admission	4 (13.3)	6 (20)	0.783
GCS of >13 at discharge	15 (50)	15 (50)	0.147
GCS of >13 at 90 days	22 (73.3)	16 (53.3)	0.032
NIHSS >9 at admission	24 (80)	23 (76.6)	0.94
NIHSS >9 at discharge	19 (63.3)	23 (76.6)	0.329
NIHSS >9 at 90 days	10 (33.3)	15 (50)	0.33
MRS <2 at 90 days	26 (86.6)	12 (40)	0.000

GCS: Glasgow Coma Scale, NIHSS: National Institutes of Health Stroke Scale, MRS: Modified Rankin Scale

Table 3: Comparison of outcome measures in two groups

Parameters	Mean (SD)		*P*
	Group 1 (intervention) (*n*=30)	Group 2 (control) (*n*=30)	
FMS (UL) at admission	28.53 (7.505)	28.27 (6.918)	0.887
FMS (UL) at discharge	29.53 (8.877)	29.53 (7.257)	1.0
FMS (UL) at 90 days	48.57 (7.408)	33.07 (7.917)	0.000
FMS (LL) at admission	11.60 (4.889)	13.77 (4.872)	0.078
FMS (LL) at discharge	12.97 (4.529)	14.63 (4.642)	0.165
FMS (LL) at 90 days	19.60 (3.4)	17.40 (4.36)	0.033
MMSE at admission	20.73 (3.503)	19.70 (3.053)	0.228
MMSE at discharge	22.60 (2.884)	20.47 (2.945)	0.006
MMSE at 90 days	26.33 (2.309)	22.77 (2.569)	0.000
MRS at discharge	4.13 (0.819)	4.10 (0.845)	0.877
MRS at 90 days	1.70 (0.794)	2.70 (9.15)	0.000
Time to achieve ADL	1.87 (0.681)	2.43 (0.626)	0.001
Total duration of hospital stay	9.53 (2.013)	9.37 (2.059)	0.752

FMS-UL: Fugl-Meyer score of upper limb, FMS-LL: Fugl-Meyer score of lower limb, MMSE: Mini-Mental Status Examination, MRS: Modified Rankin Scale, ADL: Activity of daily living, SD: Standard deviation

The Fugl-Meyer Motor Assessment Scale was assessed in upper limb (FMS-UL) and lower limb (FMS-LL) separately. The mean baseline FMS-UL at admission was 28.53 in the intervention group and 28.27 in the control group; at discharge, it was 29.53 in both the groups; and at 90 days, it was 48.57 in the intervention group while it was 33.07 in the control group which was statistically significant (P = 0.000). Figure 1 shows that till discharge, both the groups had similar scores of FMS-UL, but after discharge, recovery was better in the intervention group. The diagrammatic representation of Table 3 is shown in Figures 1-3 to highlight the change in FMS and Mini-Mental Status Examination in the two groups.

MRS was also significantly better in the intervention group as compared to controls at the end of 3 months (P = 0.000). Total 26 (86.7%) patients in the intervention group and 12 (40%) in the control group achieved the MRS of <2 (P = 0.000), and patients in the intervention group had achieved unassisted daily activities significantly earlier than control group (1.87 months vs. 2.43 months, P = 0.001). The mean duration of hospital stay was almost equal in both the groups [Table 3]. Table 3 shows the graphic representation.

Table 4 shows change in mean values of GCS and NIHSS scores in the two groups over the follow-up of 90 days.

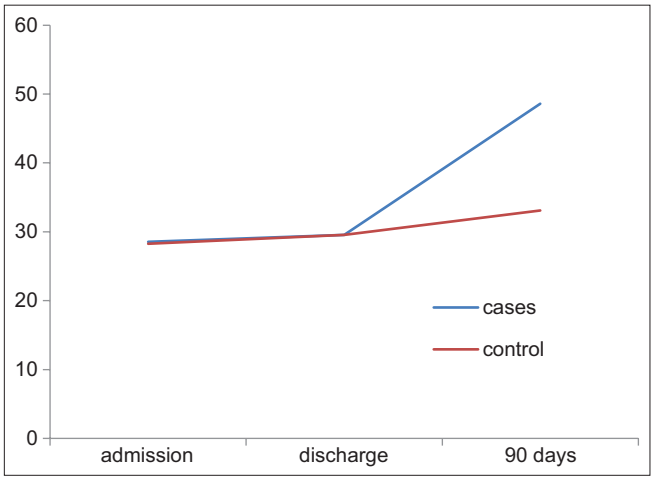

Figure 1: Mean Fugl-Meyer scale-upper limb (FMS-UL) changes over time in two groups. Figure showed rapid change in FMS-UL after discharge in intervention group (case = blue line)

Table 4: Change in mean value of the Glasgow Coma Scale and National Institutes of Health Stroke Scale scores over follow-up period of 90 days

Groups	Mean (SD)			*P*
	Value at admission	Value at discharge	Value at 90 days	
GCS in Group 1 (intervention)	10 (2.6)	12 (2.07)	13 (1.74)	0.0308
GCS in Group 2 (control)	10 (3.15)	12 (1.94)	12 (1.76)	
NIHSS in Group 1 (intervention)	15.6 (6.5)	10.3 (4.23)	7.2 (3.58)	0.0597
NIHSS in Group 2 (control)	15.4 (6.73)	12.2 (5.0)	9.2 (4.44)	

SD: Standard deviation, GCS: Glasgow Coma Scale, NIHSS: National Institutes of Health Stroke Scale

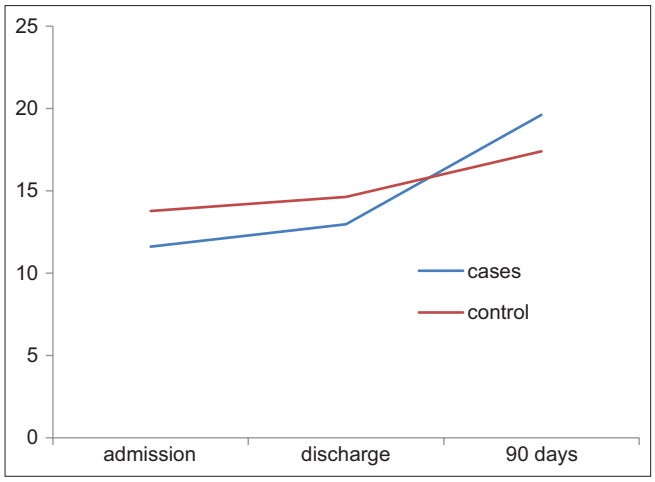

Figure 2: Mean Fugl-Meyer scale-lower limb changes over time among two groups. Almost same pace of recovery in both groups but marginally better in intervention group (case = blue line)

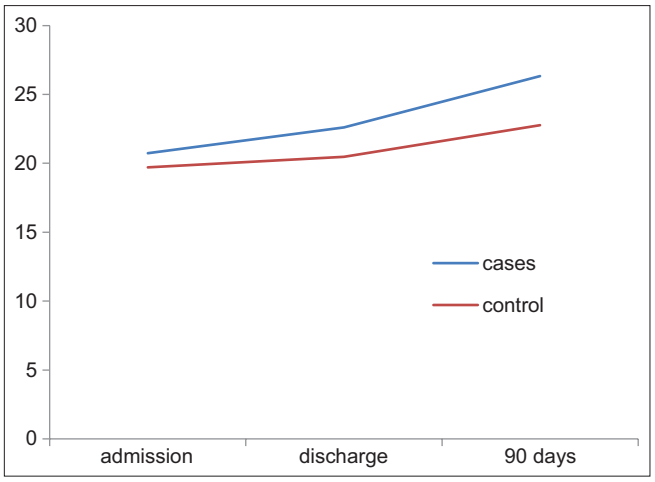

Figure 3: Mean Mini-Mental Status Examination changes over time among two groups. Better response seen in intervention (case = blue line) group

Results showed significantly better outcome in the intervention group.

Data supporting the results reported in published articles can be made available on request.

Discussion

The maximum proportion of spontaneous recovery in stroke patients usually occurs in the first 10–12 weeks; therefore, it is important to do all the efforts to achieve the best possible recovery at the end of 3 months.[26] Although the best way to minimize the poststroke disability is thrombolytic therapy (intravenous or mechanical),[27,28] due to many reasons, thrombolytic therapies are available to limited patients of acute stroke, especially in countries like India.[3,29] Ultimately, most of the patients are managed on usual medical treatment and physical therapy and wait for spontaneous recovery.

When Cheng *et al*.[7] tried to find out reasons behind failure of trials with neuroprotective agents, they postulated that factors behind unequivocal results are (1) related to tools selected for outcome scales and functional assessment of stroke, (2) heterogeneous group of patients with different premorbid conditions, and (3) difference in timing of therapeutic window.[7] Therefore, we selected a homogeneous cohort of LMCA stroke patients, and used a combination of neuroprotective agents acting through different pathways of cellular protection, within a definite time window after stroke and used an extended outcome scale for assessment of motor recovery .

The findings of the current study showed that the multimodal neuroprotection therapies acting on both "absolute" and "relative" wings of EDM, when added to standard medical treatment of acute ischemic stroke, can result in better motor and cognitive outcomes at 3 months. Motor recovery as suggested by FMS and MRS was significantly earlier and better in the intervention group. Similarly, MMSE was better in the intervention group at the time of discharge and at the end of 3 months. More number of patients had achieved the MRS of 0–2 at the end of 3 months as compared to controls.

Since revascularization therapies are available to limited number of patients of acute ischemic stroke, activation of neuronal protection, repair, and regeneration pathways remain the major focus for reducing the stroke-related disability. Astrocytes in brain contribute to stroke recovery by angiogenesis, neurogenesis, synaptogenesis, and axonal remodeling. In our study, we have targeted the "absolute pathways" of EDM by cerebrolysin (neurotrophic-like factor) and vinpocetine (PDE 1 inhibitor) and "relative pathway" by citicoline and edaravone. These molecules were having synergistic effects for neuronal repair and neuronal regeneration as their mechanism of actions is described in the following paragraphs below.

Cerebrolysin is a combination of amino acid and peptides that replicate the biological effects of neurotrophic factors. Cerebrolysin molecule had been found that multimodal and its pleiotropic action leads to immediate neuroprotection and long-term neuronal regeneration. Cerebrolysin was also found to have action at multiple levels for inducing neuronal plasticity by endogenous stem cells, with neuronal and synovial sprouting through "absolute pathway" of EDM.[15] Positive functional outcome with the use of cerebrolysin after acute ischemic stroke had been already documented in many studies.[15,25,30-33] In CARS 1 trial, cerebrolysin was found to be effective in improvement of global functional outcome when started within 72 h after acute ischemic stroke.[15] Tran *et al*. also

reported from their study that cerebrolysin, alone or in combination with other such pharmaceutical agents, provided benefit in the treatment of acute ischemia, in both the acute and recovery stages and its use was safe.[30] Various studies have shown that combining cerebrolysin with standardized rehabilitation therapy showed better motor recovery than giving standardized rehabilitation therapy alone.[31-33] About 28.5% more independent patients in the intervention group than the control group were there when cerebrolysin was used with conventional treatment.[33]

Cerebrolysin is not found to be consistently advantageous in all stroke trials and meta-analyses. Therefore, it is still not recommended in current practice guidelines.[16,17,30,34] A Cochrane review and meta-analysis by Zhang *et al*. showed that the use of cerebrolysin within 48 h of stroke was not associated with better outcome.[16,17] Heiss *et al*. conducted a double-blind placebo-controlled randomized clinical trial to assess cerebrolysin in terms of utility and safety in patients with acute ischemic stroke.[34] The validating endpoint in this trial revealed no differences between the treatment groups, but a favorable outcome trend was observed in the heavily impacted patients treated with cerebrolysin. Finally, one more meta-analysis had reported that cerebrolysin is well tolerated, associated with lower death rate but had little effects on morbidity in the intervention group.[35] In view of these equivocal results, we presume that cerebrolysin is only acting on "absolute process" of EDMs and will require the help of other agents, which acts through "relative process" of EDM for better results, as shown in our study.

Now, we will discuss the second mechanism and target for activation of "absolute pathway" of EDM after stroke. Soon after acute cerebral ischemia, there is a rise in glutamatergic transmission at cellular level which in turn leads to a rise in intracellular Ca++ influx. Calcium influx should stimulate cyclic AMP and cyclic GMP levels which are important for activation of neuroprotective cascade through cyclic AMP-responsive element binding protein (CREB) and serum response factor pathway.[18] Thus, we have another target for neuromodulation that can act through "absolute pathway" of EDM after stroke and that is the CREB protein which is a transcription factor and it plays a key role in expression of neuronal plasticity-related gene.[18] Activation of the CREB pathway can enhance the motor recovery after stroke while blocking it can prevent stroke recovery and there are evidence that PDE1 inhibitor like vinpocetine is able to increase the expression of plasticity-related gene through activation of CREB pathway.[18,19]

The cyclic AMP and cyclic GMP are rapidly converted to AMP and GMP by enzyme phosphodiesterase (PDE) at cellular level; therefore, the wanted action does not take place and we need PDEIs for blocking this conversion. PDEIs were classified into five types according to the organs they found and physiological role. Both type 1 and type 5 PDEIs are found in brain parenchyma and have an important role to increase intracellular levels of c-AMP and c-GMP. Type 1 PDEI acts on both AMP and GMP cycles, but type 5 PDEI is specific for GMP cycle. Addition of PDE inhibitors to increase the intracellular cyclic AMP and cyclic GMP can induce gene expression responsible for neuromodulation and plasticity.[6] Therefore, type 1 PDEIs (vinpocetine) found to act through upregulation of CREB pathway through cyclic AMP and cyclic GMP modulation and result in better stroke recovery.[19,20]

The role of vinpocetine in acute ischemic stroke had been reviewed in experimental and human studies, and it was proved that vinpocetine also acts though multimodal mechanisms of action like anti-inflammatory and anti-oxidative activity along with activation of CREB pathway.[20,36-42] Along with the above-mentioned mechanism, vinpocetine also acts through nuclear factor kappa B (NF-κB), voltage-gated calcium and sodium channels, interleukin-8, and tumor necrosis factor-α which are overexpressed during ischemic stroke which play a potential role in the initiation of inflammation and apoptosis.[36] Activation of NF-κB pathway leads to vascular thrombosis and augments the ischemic injury; therefore, inhibition of NF-κB pathway by vinpocetine is regarded as an important mechanism for neuroprotection.[37] Vinpocetine also inhibits the voltage-gated calcium and sodium channels and plays an important neuroprotective role after ischemic stroke.[38] Finally, vinpocetine also improves cognitive functions after stroke by improving cerebral metabolism, cerebral vasodilatation, and improvement of neurotransmitters such as serotonin, dopamine, and noradrenalin.[42] The recent systematic review and meta-analysis on vinpocetine in acute ischemic stroke included four randomized controlled trials and 601 patients and reported it to be a promising drug.[43]

After two main targets for activation of "absolute pathways," now we will see how the "relative pathways" modulation after acute stroke can be helpful for functional recovery. The first target for the "relative pathway" is an effective anti-oxidant which can be helpful for the prevention of secondary injury to neurons. Edaravone is known to be having strong free radicals scavenging action and effectively being used for neuroprotection in acute ischemic stroke.[13] As we are aware that edaravone is the only

neuroprotective agent that was approved by any national stroke guidelines (Japan) after extensive data on 61,048 patients,[13,14] as it was found to have improved outcomes after acute ischemic strokes in many studies including one study on Indian patients and three meta-analyses on stroke patients.[44-49] The survival benefit and better neurological outcome with edaravone therapy are independent of the mean age and course of treatment but better if used in Asian countries for the treatment of acute ischemic stroke.[46,47] Thus, there are a plenty of positive evidence in favor of edaravone in acute ischemic stroke as it is a potent antioxidant and acts through "relative pathway" of EDM and it can act better if used along with the agents acting on "absolute pathway' as shown in our study.

The fourth agent which was used in our study and was again working through the "relative pathway" of EDM was citicoline. Various controlled trials and meta-analyses in the recent past showed that citicoline is having variable benefits in both functional and cognitive outcomes after acute ischemic stroke.[11,12] Although the previous study had shown that citicoline is more promising in improvement of cognitive outcome as compared to motor outcome after stroke, one very recent study from India had shown that it can also reduce the infarction size although not statistically significant and had not resulted in significant improvement of NIHSS or MRS scores after acute ischemic stroke.[50,51] Mechanisms by which citicoline is activating the "relative pathway" of EDM have been described in multiple studies like stabilization of membrane integrity in neurons, glutamate inhibition, and promoting the synthesis of various proteins and neurotransmitters after acute stroke.[52-58] According to our hypothesis, citicoline alone can't work alone as it is effective only in one part of defense mechanisms.

The limitations of the study are: (1) single-center study, (2) lesser number of patients, and (3) only one type of stroke (MCA) was included. Therefore, it requires a larger sample size with multicentric study to confirm the results.

Conclusion

Though our pilot study with small number of patients is having limitations but it supports the hypothesis that activation of both "absolute and relative pathways" of EDM can be a better option and hope for the future for improvement in motor and cognitive outcomes after large-vessel acute ischemic stroke. In future multicentre studies with larger sample size are required to confirm these results.

Author contributions

Dr. Goel: Concept & design, data acquisition, analysis and interpretation of data, review of literature and manuscript preparation; Dr. Shangri: Data acquisition, analysis and interpretation of data, review of literature and manuscript preparation; Dr. Mittal: Concept & design, analysis and interpretation of data, review of literature and manuscript preparation; Dr. Bhat: Analysis and interpretation of data, review of literature and manuscript preparation.

Ethical statement

The study was approved by the Ethics Committee of Swami Rama Himalayan University (No.: ECR/483/Inst/UK/2013/RR-16, dated on August 13th, 2020) and has been registered with clinical trials registry (ID: REF/2017/08/015183).

Declaration of Helsinki

We declare that all essential components of declaration were followed in the current study. The health of our patients will be our first consideration and we will act in the patients' best interest when providing medical care during research on human subjects.

Patients consent

Informed consent was taken from each of the patient before enrolment.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Financial support and sponsorship

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Conflicts of interest

There are no conflicts of interest.

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