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Can Neuroprotective Polytherapy Result in Better Neurological Outcome in Acute Large Middle Cerebral Artery Ischemic Stroke? A Case Control Study

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Abstract

Background: *Stroke is a leading cause of morbidity and mortality worldwide. A small number of patients have access to timely acute stroke interventions like thrombolysis or thrombectomy which may halt or reverse the injury. The remaining patient use to have a protracted and incomplete recovery of motor and cognitive functions. Neuroprotective therapies usually been evaluated and administered singularly have not been found successful so far in ameliorating post stroke disability. Hence currently none of the neuroprotective agent approved American, European, and Indian stroke guidelines. Our hypothesis is that combining neuroprotective agents with different mechanisms can be useful.*

Aims and Objectives: *To study the effect of combination of neuroprotection therapies in acute Large Vessel Middle Cerebral Artery [LMCA] ischemic stroke.*

Methods: *A randomized controlled trial, comprising 60 patients of acute Middle Cerebral Artery [MCA] infarction who presented to the hospital within 72 hours and were randomly distributed into two groups of 30 patients each. The control group received Standard Medical Care while the intervention group received Standard Medical Care combined with Cerebrolysin , Edaravone, Vinpocetine, Levetiracetam and Citicoline, started within 72 hours of onset of stroke .Patient were assessed on basis of NIHSS, Fugl Meyer Assessment score, Glasgow Coma Scale , MMSE at admission , discharge and after 90 days.*

Results: *The intervention group showed significant improvements in motor recovery as measured by the Fugl-Meyer scores, better Modified Rankin Scale scores and better cognitive recovery as observed by MMSE scores.*

Conclusion: *Combination therapy for neuroprotection with multimodal actions may provide improvements in motor and cognitive recovery when initiated within 72 hours of ischemic stroke onset. However, large scale studies are warranted to solidify these findings.*

Keywords: Neuroregenerative Therapy in Stroke, Neuroprotection in Stroke, PDE Inhibitors in Stroke

Introduction

Stroke is the most common global cause of death and disability all over the world [1]. Due to better acute care and high survival rate, stroke is now considered as a chronic neurological condition rather than monophasic acute illness. [2]. Due to chronic disability, stroke related global burden of disease is very high [3]. Even after best acute stroke related care more than half of survivors have to live with one or more functional disabilities for the rest of their lives [2]. Most of the stroke survivors lose at least one function [motor, sensory or cognitive] of their pre-stroke life [2]. In one study on Indian population, persistent disability among stroke survivors was mild in 42.4%, moderate in 43% and severe in 14.6% respectively [4]. Disability and mortality is more prominent in patients with large vessel stroke.

Most of the patients shows maximum recovery in first 10-12 weeks after acute stroke [5]. Therefore, it is important to do all the efforts with both medical treatment and rehabilitation to achieve best possible recovery within 10 – 12 weeks of acute stroke. Among currently available therapies, the best tool to reduce post stroke disability is thrombolytic therapy [intravenous or mechanical] within specified window period after acute stroke

[6, 7]. However, the limitation of thrombolytic therapy is that, it is practically applicable in less than 10% of acute stroke patients especially in Indian setup due to various reasons [3, 8]. Even after thrombolysis only one third patients get reversibility, thus most of the patients wait for spontaneous recovery after standard medical treatment and physical therapy. Question is that can we augment the process of repair by endogenous neuro-modulation or neuro-regenerative mechanisms?

Various neuro-protective therapies tried so far for promoting neuronal repair or regeneration are growth factors, monoclonal antibodies, drugs, cell-based therapies, activity-based therapies, and brain stimulation-based therapies. [9]. In last two decade many efforts have been made to evaluate various neuro-protective pharmacological agents that can alter the course of stroke recovery and results in better neurological outcome [10, 11]. Common neuroprotective molecules tested for clinical usage were Selective Serotonin Reuptake Inhibitors [SSRI] [12-14], Citicoline, Edaravone, Cerebrolysin, and Phosphodiesterase Inhibitors [PDEI] [24].

Unfortunately, even after extensive efforts in last two decades, most of the trials on neuro-protective molecules failed to show unequivocal and consistent improved functional outcome after stroke [11]. Therefore, American, European and Indian stroke guidelines do not recommend the routine use of neuro-protective agents in management of acute ischemic stroke patients [1, 25].

Failure of neuroprotective trials was critically analyzed to find out the factors behind negative results [11]. Many discrepancies were highlighted which had resulted in the failure of trials on neuro protection treatment [11]. Some of the important observations that could explain the negative results with neuro protection were; 1] related with 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 [11]. In most of the studies efficacy of neuro-protective agents was judged by; 1] reduction of infarction volume; 2] NIHSS [national institute of health stroke scale]; and 3] modified Rankin scale [MRS]. But as we know, the infarction volume was poorly correlated with functional deficit in stroke patients and a small lesion at critical position can cause major functional deficit [11]. Outcome scale used in most of the studies on neuro-protection was Modified Rankin scale [MRS] at 90 days, is a very crude method for assessment of motor, sensory or cognitive functions after stroke and options like Fugl-Meyer and Motor Assessment Scale [MAS] can reflect functional outcome in better way after acute stroke. Moreover, post-stroke cellular injury cascades are related to multiple molecular mechanisms for neuronal death and poor expression of neuronal plasticity, like inflammation, oxidative stress, excitotoxicity, mitochondrial failure and other failure of transcription pathways responsible for neuro-modulating gene expression. [26, 27]. Therefore, using single molecule which is acting through single mechanism to prevent neuronal injury might not show adequate response to change the post stroke functional outcome. If we can simultaneously act on different arms to control inflammation, oxidative stress, glutamate excitotoxicity, and expression of neuromodulation gene then probably we can get better results.

Based on above background we hypothesized that, if one selects homogeneous cohort of stroke patients, with use of combinations of neuro-protective agents acting through different pathways of cellular protection, in definite time window after stroke and using better outcome scale for assessment of motor recovery can give better functional outcome. Recently researchers felt that neuroprotection therapies with multimodal action can be the best option to reduce the stroke related disability and they recommended that multimodality of neuroprotection in stroke should be systematically and intensively investigated for future advancement [28].

Therefore, we have conducted this pilot study on selective group of acute ischemic stroke in which we had given combination of five neuro-protective agents acting through different mechanism of action within 72 hours of acute ischemic stroke. This study was done to analyze the motor outcome at 12 weeks after acute stroke with combination of neuro-protective agent supplementation.

Methods

Aim of t he study was to find out effectiveness of Neuroprotective Polytherapy in motor outcome after first acute large vessel middle cerebral artery [LMCA] territory cortical infarction. Study design was randomized controlled study. Method for randomization used was simple randomization technique. Patients with first attack of acute LMCA ischemic stroke with National Institute of Health Stroke Scale [NIHSS] score of ≥ 6 were enrolled and randomized to two groups; Group1: it was intervention group in which patients presenting with First attack of Acute LMCA infarction within 72 hours of onset and put on standard medical stroke treatment according to Indian Stroke Guidelines, along with Multimodal Neuro-protective polytherapy. Group 2: it was control group in which patients presenting with First attack of Acute LMCA infarction within 72 hours of onset and put on standard stroke treatment according to recommendation of Indian Stroke Association 2018, without any Neuro-protective agents; the study design was approved by institutional ethical committee and written consent will be obtained by the subject [1].

Definitions

LMCA cortical Infarction: Radiological evidence of acute cortical involvement of large vessel Middle Cerebral Artery [LMCA] territory infarction on radiological imaging.

First Attack: there should be no clinical as well as radiological evidence of prior ischemic or hemorrhagic stroke. Within 72 hours: will be taken from the time last seen normal. In case of wake stroke last seen normal will be the time last seen normal before going to sleep..

Patients of LMCA stroke not fitting in selection criteria, not giving the consent, having serious co-morbid illness like kidney failure on haemodialysis, cancers, already chair/bed bound and disabling psychiatric illness like Dementia or Parkinson's disease, who have complete or partial reversal after thrombolysis, and minor stroke [NIHSS ≤ 5], and small vessel lacunar stroke were excluded from study.

Neuro-protective polytherapy: Combination of Five Agents acting on different arm of Patho-physiological cascade of acute ischemic stroke; 1] Injection Cerebro-protein 60 mg daily for 7 days IV Infusion; 2] Injection Edaravone 60 mg daily for 7 days

IV infusion; 3] Injection Levetiracetam 500 mg twice daily for 7 days; 4] Vinpocetine 10 mg daily oral route for 12 weeks and 5] Citicoline 500 twice daily orally for 12 weeks. All neuro-protective agents were started within 72 hours of acute stroke.

Severity of stroke: assessment done with Glasgow coma scale [GCS], National institute of health stroke scale [NIHSS], and mini mental status examination [MMSE] at admission done to assess severity.

Primary Outcome measure: Measured by Modified Rankin Scale [MRS], at discharge and 90 days along with Fugel Mayer scale [FMS] in upper and lower limb at 0, day of discharge, 30 days and 90 days were seen 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 time to achieve this unassisted pervious daily activity.

Statistical analysis: It was done with IBM SPSS -25 and Fisher Exact Test, chi square test for group and Student "t" test for the mean was used for statistical analysis.

Results

Total 147 patients of new onset ischemic stroke were admitted in one year [November 2021 to October 2022], out of them 89 [60.5%] patients had anterior circulation stroke and 77 [52.4%] had middle cerebral artery [MCA] territorial involvement. Total 17 patients, 12 patients [15.6%] died and 5 [6.5%] lost to follow-up [5]. Total 60 patients enrolled in this pilot study [30 in control [group 1] and 30 in the intervention arm [group 2]. Demography and profile of these patients is given in table 1.

Table 1: General demographic parameters in two groups

Parameters	Group1 [cases]	Group 2 [control]	P value
	N= 30	N = 30	
Gender	Male = 20 [67%]	Male = 21	0.5
	Female = 10 [33%]	Female = 9	
Diabetes	Yes = 13	Yes = 14	0.5
	No = 17	No = 16	
Hypertension	Yes = 18	Yes = 18	0.603
	No = 12	No = 12	
Smoking	Yes = 17	Yes = 17	0.603
	No = 13	No = 13	
Alcohol	Yes = 7	Yes = 8	0.5
	No = 23	No = 22	
Cardiac cause for stroke	Yes = 6	Yes = 6	0.5
	No = 24	No = 24	
Atrial fibrillation	Yes = 6	Yes = 4	0.365
	No = 24	No = 26	
Carotid artery stenosis of > 50%	Yes = 19	Yes = 17	0.396
	No = 11	No = 13	
Low Density Lipoprotein [LDL] level > 150 mg/dl	Yes = 22	Yes = 17	0.139
	No = 8	No = 13	
Hyperhomocystinemia	Yes = 8	Yes = 8	0.614
	No = 22	No = 22	
Side of stroke	Right *MCA = 13	Right MCA = 12	0.5
	Left MCA = 17	Left MCA = 18	

MCA = Middle cerebral artery

Table 1 showed that both the groups had homogenously distributed parameters. Both the groups had similar gender ratio with 2/3rd male population, 45% of patients had diabetes in both groups, about 60% had hypertension and were active smokers, 25% patients had habit of daily alcohol consumption, 21.6% patients had underlying cardiac cause of embolization, similar number had underlying atrial fibrillation, 60% patients also had more than 50% carotid artery stenosis, 65% patients had high low density lipoprotein [LDL] levels and 26.6% had Hyperhomocystinemia as a risk factor of stroke. About 41% patients had right middle cerebral artery [MCA] and 59% patients had left MCA stroke.

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

Parameters	Group 1 [cases]	Group 2 [control]	P value
GCS at admission	GCS > 13 = 4	GCS > 13 = 6	0.783
	GCS 9-12 = 17	GCS 9-12 = 16	
	GCS < 8 = 9	GCS < 8 = 8	
GCS at Discharge	GCS > 13 = 15	GCS > 13 = 15	0.147
	GCS 9-12 = 10	GCS 9-12 = 10	
GCS at 90 days	GCS > 13 = 22	GCS > 13 = 14	0.032
	GCS 9-12 = 8	GCS 9-12 = 16	
NIHSS at admission	NIHSS < 6 = 0	NIHSS < 6 = 0	0.94
	NIHSS 6-8 = 6	NIHSS 6-8 = 7	
	NIHSS 9-15 = 11	NIHSS 9-15 = 10	
	NIHSS > 16 = 13	NIHSS > 16 = 13	
NIHSS at Discharge	NIHSS < 6 = 0	NIHSS < 6 = 0	0.329
	NIHSS 6-8 = 11	NIHSS 6-8 = 7	
	NIHSS 9-15 = 15	NIHSS 9-15 = 15	
	NIHSS > 16 = 4	NIHSS > 16 = 8	
NIHSS at 90 days	NIHSS < 6 = 9	NIHSS < 6 = 4	0.33
	NIHSS 6-8 = 11	NIHSS 6-8 = 11	
	NIHSS 9-15 = 8	NIHSS 9-15 = 10	
	NIHSS > 16 = 2	NIHSS > 16 = 5	
MRS at 90 days	MRS 0-2 = 26	MRS 0-2 = 12	0.000
	MRS ≥ 3 = 4	MRS ≥ 3 = 18	

Table 2 shows dynamic changes in stroke severity parameters over 90 days follow up. At the time of admission only 4 [13.3%] patients in intervention group had Glasgow coma scale [GCS] of more than 13 while at the end of 3 months 73.3% had GCS of > 13, thus the intervention group had significantly high- er recovery of GCS at 90 days [p=0.032] as compared to control group. At the end of 3 months intervention group had higher proportion of patients with < 6 NIHSS [national institute of health stroke scale] which was not statistically significant [30% versus 13.3%, p = 0.33]. Significantly high proportion of patients had Modified Rankin Scale [MRS] of 0-2 [minimal disability] in intervention group at 90 days [p = 0.000].

Table 3: comparison of outcome measures two groups

Parameters	Mean [SD] in Group 1 [cases]	Mean [SD] in Group 2 [control]	P value
	[n = 30]	[n = 30]	
Age	60.07 [12.180]	60.37 [12.813]	0.926
FMS [UL] at admission	28.53 [7.505]	28.27 [6.918]	0.887
FMS [UL] at discharge [24]	29.53 [8.877]	29.53 [7.257]	1.0
FMS [UL] at 90 days [25]	48.57 [7.408]	33.07 [7.917]	0.000
FMS [LL] at admission [26]	11.60 [4.889]	13.77 [4.872]	0.078
FMS [LL] at discharge [27]	12.97 [4. 529]	14.63 [4.642]	0.165
FMS [LL] at 90 days [28]	19.60 [3.4]	17.40 [4.36]	0.033
MMSE at admission [29]	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 [31]	26.33 [2.309]	22.77 [2.569]	0.000
MRS at discharge [32]	4.13 [0.819]	4.10 [0.845]	0.877
MRS at 90 days [33]	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 = Fugel-Meyer Score of Upper Limb
FMS-LL = Fugel Meyer Score of Lower Limb
MMSE = Mini Mental Status Examination
MRS = Modified Rankin Scale
ADL = Activity of Daily Living

Table 3 showed the changes in motor, cognitive and overall disability related outcomes at the end of 3 months. Fugel-Meyer motor assessment scale was assessed in upper limb [FMS-UL] and lower limb [FMS-LL] separately. Mean baseline FMS-UL at admission was 28.53 intervention group and 28.27 in control group, at discharge it was 29.53 in both groups and at 90 days it was 48.57 in intervention group while it was 33.07 in control group which was statistically significant [p = 0.000]. Figure 1 showed that till discharge both the groups had similar scores of FMS-UL but after discharge recovery was better in intervention group.

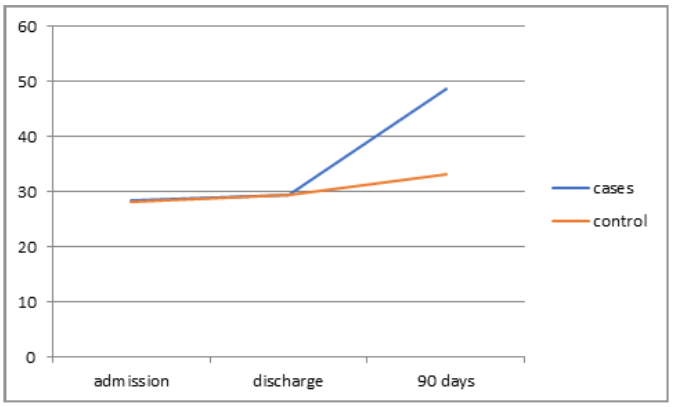

Figure 1: Mean Fugel Meyer scale - Upper limb FMS-UL changes over time in two groups

Mean FMS-LL score at admission and discharge was comparable in both groups but at the end of 3 months score was significantly better in intervention group [19.6 versus 17.4, p = 0.033]. Figure 2 is showing that trajectory of FMS-LL was better in intervention group.

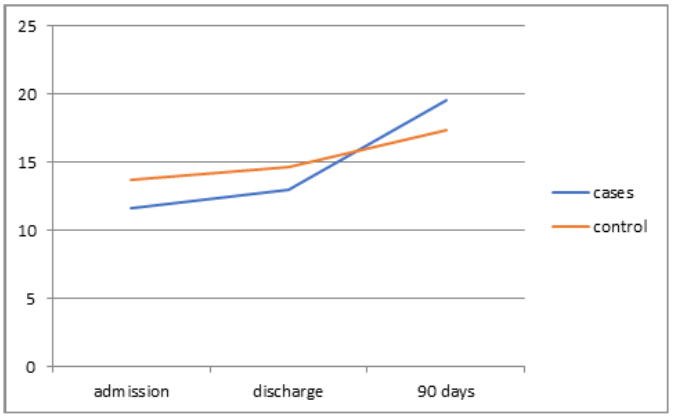

Figure 2: Mean Fugel Meyer scale – lower limb (FMS-LL) changes over time among two groups

Table 3 and figure 3 show that cognitive improvement was also better in intervention group at the end of 3 months assessed by MMSE scores. MMSE scores were significantly higher in intervention group at the time of discharge [p = 0.006] and at the end of 3 months [p = 0.000].

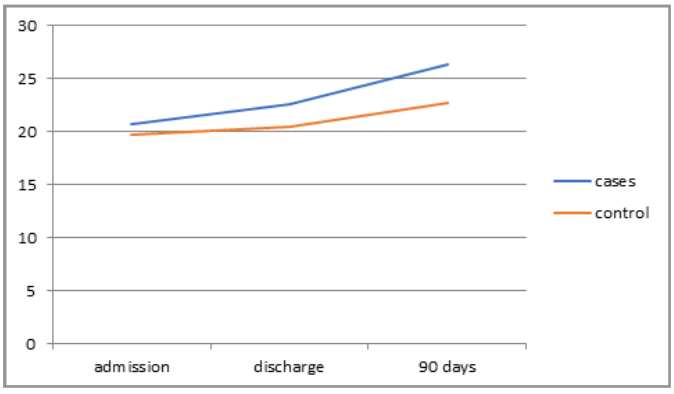

Figure 3: Mean mini mental state examination (MMSE) changes over time among two groups

Modified Rankin Scale [MRS] was also significantly better in intervention group as compared to control at the end of 3 months [p = 0.000]. Total 26 [86.7%] patients in intervention group and 12 [40%] in control group achieved the MRS of < 2 [p= 0.000] and patients in intervention group had achieved unassisted daily activities significantly earlier than control group [1.87 month versus 2.43 months, p = 0.001]. Mean duration of hospital stay was almost equal in both groups [table 3].

Discussion

Findings of current study showed that the multimodal neuroprotection therapies with different mechanism of action when added to standard medical treatment of acute ischemic stroke can result in better motor and cognitive outcome at 3 months. Motor recovery as suggested by FMS and MRS was significantly earlier and better in intervention group. Similarly, MMSE was better in 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 three months as compared to control.

After acute stroke, the cascade of primary and secondary neuronal injury begins along with the activation of endogenous defense pathway which is responsible for minimizing the insult. Activation of Endogenous Defense [ED] pathway results in restoration of neuron by following three mechanisms 1) neuronal protection, 2) neuronal repair and 3) neuronal regeneration [28]. ED itself consists of two important processes, one is absolute and other is relative [28]. The absolute process of ED leads to gene expression and protein synthesis which helps for neuronal restoration.Neurotrophic factors and Neurotrophic like molecules control the absolute mechanisms. Other relative process of ED is dependent on agonists/antagonists for various ion channels and receptors with anti-inflammatory, anti-oxidative and anti-excitotoxin activity for neuronal protection [28]. There are three major mechanisms which lead to secondary neuronal injury after primary and direct neuronal damage after acute ischemic stroke; 1] excitotoxin related neuronal injury; 2] excessive oxidative stress after vascular insult; and 3] local immune response leading to inflammation at local tissue after acute tissue injury [29]. Finally, all primary and secondary insults after acute stroke activates the last mechanism for neuronal death, that is activation of apoptosis pathway.

Revascularization therapies are the important way to prevent or minimize the amount of direct neuronal damage. As most of the patients do not qualify for the revascularization therapies, promoting the neuronal repair and neuroregeneration is the best option for recovery. Therefore, neuronal protection, repair and regeneration remain the major focus for reducing the stroke related disability. Astrocytes in brain contribute to stroke recovery by angiogenesis, neurogenesis, synaptogenesis, and axonal remodeling. Therefore, Astrocyte is a designated target for neuronal protection therapies and later stroke recovery. Astrocytes limit lesion extension by preventing anti-excitotoxicity effects by releasing neurotrophins [29]. If we need multimodality of neuroprotection in stroke then ideal forms of therapies would be that if it can limit the excitotoxicity, oxidative stress and inflammation through relative process of ED on one hand and also act to improve neuronal repair and also can activate the plasticity related pathway through absolute process of ED.

In our study we have targeted multiple pathways which are involved in primary and secondary neuronal injury after stroke. Cerebrolysin [neurotrophic like factor] and Vinpocetine [PDE 1 inhibitor] were primarily responsible for the absolute process of ED while Levetiracetam [SV2A inhibitor], Citicoline and Edaravone were responsible for relative process of ED.

Cerebrolysin is a combination of amino acid and peptides that replicates the biological effects of neurotrophic factors. Cerebrolysin molecule had been recently reviewed by Muresanu et al [11] and found that multimodal and pleiotropic action of Cerebrolysin leads to immediate neuroprotection and long term neuronal regeneration. Cerebrolysin also found to have action at multiple levels for inducing neuronal plasticity by endogenous stem cells, anti-inflammatory effects, neuronal and synovial sprouting [11]. Cerebrolysin was also found effective in global functional outcome if started within 72 hours after acute ischemic stroke in CARS 1 Trial [19]. Although in Cochrane review the use of Cerebrolysin within 48 hours of stroke was not found to be associated with better outcome [20]. Another meta-analysis also found no definite advantages of Cerebrolysin in acute ischemic stroke. [21]. Next study CARS 2 was conducted in 2015 but it could not support the findings of CARS 1 [30]. Tran et al. [31] 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 [31]. Chang et al. [32] performed a study combining Cerebrolysin with standardized rehabilitation therapy. They showed that in patients with severe motor impairment caused by acute ischemic stroke, conventional rehabilitation therapy combined with Cerebrolysin provided additional benefits to conventional rehabilitation therapy alone in motor recovery [32]. Razei et al. [33] conducted a randomized, double-blinded, placebo-controlled trial which focused on Cerebrolysin and their results suggested that Cerebrolysin might be beneficial for patients with acute focal ischemic stroke and improves their neurological results, also affecting the pulsatility index [PI] of the middle cerebral artery. Stan et al. [34] analysed the efficacy of Cerebrolysin combined with post-stroke early rehabilitation, showing positive results in the cohort treated with Cerebrolysin. The authors reported improved overall neurological health and reduction in the impairment for the patients treated with Cerebrolysin as there were 28.5% more independent patients in the intervention group than the control group, emphasizing that this study's positive findings [34]. Heiss et al. [35] conducted a Double-blind placebo-controlled randomized clinical trial [529 Cerebrolysin and 541 placebos] to assess Cerebrolysin in terms of utility and safety in patients with acute ischemic stroke. The validating endpoint in this trial revealed no differences between the treatment groups but a favourable outcome trend was observed in the heavily impacted patients treated with Cerebrolysin. A meta-analysis of 9 RCTs had reported a lower death rate in Cerebrolysin group of patients and final conclusion of all trials and meta-analysis was that Cerebrolysin is well tolerated and can reduce mortality but having little effects on morbidity when used alone in acute ischemic stroke [36]. We presume that Cerebrolysin is only helping the absolute process of endogenous defense mechanisms and will require help of other agents which acts through relative process of EDA for better results.

Another target for neuro modulation after stroke is Cyclic-AMP-Response-Element-Binding [CREB] protein, a

tran-scription factor that plays a key role in expression of neuronal plasticity related gene [22]. Increasing levels of CREB enhances the motor recovery after stroke and blocking CREB signaling prevents stroke recovery [22]. Medina et al explored the path- way by which PDE1 inhibitors are able to increase expression of plasticity related gene through CREB [22, 23]. Along with CREB, low expression of synaptic voltage gated potassium channel pathway [Kv2.1] leads to high resistance against apoptosis and low activation of synaptic NMDA activates NSCs to exert a neuroprotective response [29]. Therefore, if we can augment the CREB activity and lower the Kv2.1 and NMDA activity we can have better neuronal modulation. Phosphodiestrase Inhibitor [PDEI] was classified into five types according to organs they were found in and physiological role. Both type 1 and type 5 PDEI are found in brain parenchyma and have important role to increase intracellular levels of c-AMP and c-GMP. Type 1 PDEI acts on both AMP & GMP cycles but type 5 PDEI is specific for GMP cycle. Most of the previous studies had explored the role of Vinpocetine, a PDE1 inhibitor in stroke patients [24]. Type 1 PDEI, acts specifically through up-regulation of CREB pathway through cyclic AMP and cyclic GMP modulation and are useful for stroke recovery (Figure 4) [23].

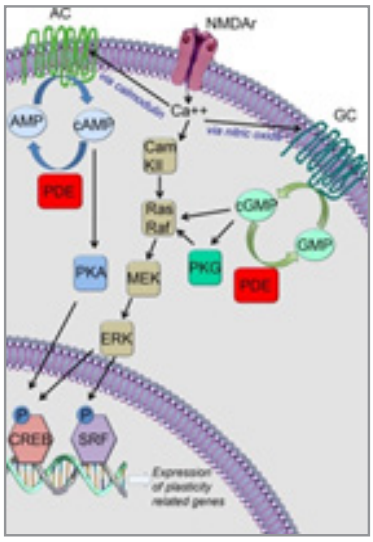

Figure 4: CREB pathway (adopted from Medina AE et al. 2011 [23].

After acute cerebral ischemia there is rise in glutaminergic transmission at cellular level which in turn leads to rise in intracellular calcium influx. Calcium influx should stimulate c-AMP & c-GMP levels which are important for activation of neuro-protective cascade through c-AMP responsive element binding protein [CREB] and Serum response factor [SRF] pathway. The c-AMP and c-GMP are rapidly converted to AMP and GMP by PDE at cellular level therefore the wanted action does not take place. Addition of PDEI as an agent to increase the levels of c-AMP and c-GMP can induce better gene expression which helps in neuro-modulation and plasticity. This hypothesis has been proved in many experimental and clinical studies [10].

Role of Vinpocetine in acute ischemic stroke has recently being reviewed by Hayder Al M Kuraishi et al [24] in experimental & 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. As we know that Vinpocetine is a selective inhibitor of Phosphodiestrase type 1 [PDE1] which increases the concentration of cAMP and cGMP, NF-kB along with Interleukin-8[IL-8], and tumour necrosis factor [TNF-α] are over expressed during ischemic stroke which play a potential role in the initiation of inflammation and apoptosis [37]. Vascular smooth muscle and endothelial cells of cerebral vasculature are activated by NF-kB pathway leading to further obstruction and thrombosis. Therefore, NF-kB pathway is an important pathway in the pathogenesis and development of neurological deficit thus, inhibition of NF-kB pathway by Vinpocetine is regarded as important mechanism for neuroprotection [38]. During ischemic stroke, voltage gated sodium channels are activated causing intracellular accu- mulation of Sodium and Calcium leading to neuronal cell damage, excitotoxicity, oedema, acidosis and acute cellular dysfunctions. Vinpocetine inhibits voltage gated sodium channels leading to dose dependent reduction of intracellular concentrations of sodium and calcium. Thus, the neuroprotective effect of Vinpocetine during ischemic stroke is also mediated by inhibition of neuronal voltage gated sensitive Na-channel [39]. Multiple studies and literature have illustrated that oxidative stress, excitotoxicity and impaired energy metabolism culminates into neuronal death by both apoptosis and necrosis during ischemic stroke. This causes reduction of cAMP system which is important in the expression and regulation of brain derived neurotrophic factor [BDNF], which improves neuronal survival. PDE1 is mainly localized in striatum and cortex which participates in the regulation of neuronal motor activity [40, 41]. Vinpocetine increases neuronal cGMP through inhibition of calmodulin dependent Phosphodiestrase which improves cerebral blood flow and oxygen consumption [42]. Vinpocetine improves cerebral metabolism by improving glucose and oxygen supply and ATP production by cerebral vasodilatation. This prevents ischemic stroke induced-memory and cognitive dysfunctions due to improvement of neurotransmitters such as serotonin, dopamine and noradrenaline, which are involved in the regulation of cognitive function [43].

Levetiracetam [SV2A inhibitors] for 7 days not only acts as antiepileptic but was used as anti-glutamate agent by SV2A blocking action at synaptic cleft. The blockage of excitotoxin at neuronal end will help in reducing damage after ischemic cascade. SV2A inhibitors are mainly projected in epilepsy management and the multiple proposed mechanisms are through anti-glutaminergic, anti-inflammatory, anti-oxidant and neuroprotective actions which are again effective in acute stroke recovery [44]. As we know that both clinical and subclinical seizures in acute phase can results in bad neurological outcome of stroke, SV2A inhibitors along with neuroprotective effects as mentioned earlier, but also have indirect protection by managing obvious and subtle seizures during 1st week after acute MCA stroke. Previous studies have shown that patients with large vessel ischemic stroke can have obvious [4%] seizures as well as non-convulsive seizures [up to 30-40%] in 1st week of illness [45, 46]. Both obvious and subtle acute symptomatic seizures can increase the mortality and morbidity of acute ischemic stroke [45]. Thus, SV2A inhibitors in 1st week of acute ischemic stroke can be helpful with direct and indirectmechanisms. Both PDE 1 inhibitors and SV2A inhibitors can be helpful for down regulation of NMDA thus can help for the neuronal stem cells mobilization and neuronal regeneration.

Edaravone is a strong free radicals scavenger used for neuro-protection in acute ischemic stroke [17]. Till date, Edaravone is the

only neuro-protective agent could get approval by international stroke guideline [Japanese Guidelines 2015, level B approval] [18]. Edaravone helps improve outcomes in ischemic strokes by acting as a scavenger of hydroxyl, peroxyl, and superoxide-free radicals, thus decreasing cerebral edema, and also inhibiting delayed neuronal death [47, 48]. A recent meta-analysis of seven randomized controlled trials and pooled data of 2069 patients indicated that Edaravone can retard neurological impairment with a survival benefit at three-month follow-up, irrespective of the mean age and course of treatment [49]. The author in this meta-analysis had concluded that Edaravone can improve neurological improvement with a survival benefit at three months follow-up, regardless of the mean age and course of treatment. He further suggested that Edaravone should be promoted for usage in Asian countries for the treatment of acute ischemic stroke [49]. A randomized controlled trial from a north Indian centre including 50 patients concluded that Edaravone effectively improved functional outcome in Acute Ischemic Stroke at 90 days [50]. A retrospective cohort study with data obtained from Japan on 61,048 ischemic stroke patients concluded that for all stroke subtypes [large-artery atherosclerosis, cardioembolic, and small-vessel occlusion], Edaravone had a statistically significant beneficial effect on neurological symptoms, although the difference was small and of limited clinical significance [17]. Recently another meta-analysis in year 2022 also found Edaravone as a promising drug in acute ischemic stroke [49, 51]. The Edaravone is the powerful agent acting through relative ED pathways and if it act together with absolute ED activation it can give further improvement in stroke outcome which had shown in our study.

Citicoline was another drug extensively studied and showed variable benefits in both functional and cognitive outcome after stroke [15]. Citicoline provides stability to cell membranes by increasing phosphatidylcholine and sphingomyelin synthesis [52, 53]. It also checks the release of free fatty acids [54]. By maintaining the integrity of the membranes, Citicoline inhibits glutamate release during ischemia. Citicoline has been suggested to decrease glutamate levels and stroke size [55]. Citicoline promotes the synthesis of nucleic acids, proteins, acetylcholine and other neurotransmitters, and inhibits free radical formation. Citicoline has also been suggested to enhance synaptic outgrowth and increased neuroplasticity [56, 57].It also improves the neurologic deficits, behavioural performance, learning and memory tasks [58]. Recent meta-analysis to study role of Citicoline in acute ischemic stroke indicates some benefit in patients who could not be thrombolised for acute ischemic stroke. [16]. Citicoline is a molecule which was found to have some benefits in one meta-analysis in year 2016 [16]. Citicoline is more promising in improvement of cognitive outcome as compared to motor outcome after stroke [59]. One recent study from All India Institute medical sciences Delhi, India had also shown that Citicoline can reduce infarction size and improvement in NIHSS score in patients of stroke [60]. This study has created new hopes with this molecule as now we have better evidences for improvement in motor outcome when used early in acute stroke. Citicoline is the only molecule which has shown positive long-term outcome in cognitive functions as well as role in reduction of infarction size, thus inclusion of this molecule in our multimodal regimen is justified.

Conclusion

It is obvious that combination of the neuroprotective agents working on different pathways of endogenous defense mechanisms can be a good option to reduce disability after first large vessels MCA stroke. Our study though limited in number, is one of the first of its kind which involves the simultaneous use of different possible neuroprotective therapies targeting separate pathways of damage and repair. These drugs are easily available and not very expensive. No special administration techniques are required. The study showed possible albeit limited motor and cognitive improvements in patient who received the treatment. However, it also highlighted the possibility that a window of opportunity may still exist for those patients who are not fortunate enough to have timely access to a stroke centre to undergo thrombolysis or Thrombectomy. The pathophysiology of damage and repair may continue for extended periods of time which may be tapped for accelerating patient recovery and improving the patient's quality of life. Finally, the combination of neuroprotective agents which can act through different mechanism and having multimodal neuronal protection and regenerative activity can results in better outcome in acute ischemic stroke with large vessels occlusion. Secondly, to study the role of neuroprotective agents in stroke patients should be evaluated with more detail outcome measures like FMS and MAS rather than only MRS. We recommend planning of a large-scale study in order to further confirm our results and inculcate them in routine practice and standard of care.

Author Contributions

DG and YS contributed to concept and design of the study, SS, DG organized the database, MM and AB done the critical literature search, DG performed the statistical analysis, SS wrote the first manuscript, and all authors had reviewed different part of manuscript with all correction and final draft development.

Conflicts of interest

The authors declare that there is no conflict of interest

Ethical approval

The study was approved by the research and ethical committee of institute (ref number: HIMS/RC/2020/241).

Consent of participants

Informed consent to participate in the study was obtained from all participants.

Consent to publication

Informed consent to publication was obtained from relevant participants.

Availability of data

"The datasets this study can be found in the [New Data on stroke Protection] [NEUROPROTECTION.xlsx]."

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