An Effective Neuroprotective Treatment in Ischemic Stroke and Cerebral Trauma with Low Doses of L-Arginine, Lamotrigine and Tianeptine

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Abstract

In stroke and cerebral trauma the damaged neurons release Aspartate and Glutamate that contribute to neuronal death (excitotoxic cell death). However, physiological levels of NMDA receptor activity can promote neuronal survival and resistance to trauma, which explain why a large number of neuroprotective agents development for stroke have failed to show positive effects in Phase III trials in ischemic stroke. The levels of free serotonin (5-HT) increase in thrombotic events, worsening the platelet aggregation and the arteriolar vasoconstriction. Endothelium-derived Nitric Oxide (eNO) is reduced in ischemic stroke. The objective of this study was to evaluate the neuroprotective effects of Lamotrigine (a glutamate release inhibitor), Tianeptine (reducer of 5-HT levels) and L-Arginine (precursor of eNO) at low doses. We performed a controlled study with 49 patients with cerebral trauma and 25 patients with ischemic stroke. We compared the sample groups with control groups that received conventional treatment. To evaluate the disease progression we used the Glasgow Scale and NIHSS. The results were analyzed by F statistical test and Student’s t test with p ≤ 0.05. The results show that patients with stroke and brain trauma are benefited with this new neuroprotective treatment (p value < 0.05) with lower rates of neurologic complications. Key Words: Glutamate, NMDA-receptor signalling, free serotonin, endothelial Nitric Oxide, Agmatine, Pro-survival signal, Neurotoxicity.

Cerebrovascular Diseases (CD), the third leading cause of death in developed countries after heart diseases and cancer, has an overall prevalence of 795 per 100,000 and are a major cause of disability. Two thirds of stroke survivors suffer from residual neurological deficits and have to cope with chronic motor and language dysfunctions. So far, we have very limited effective therapies in spite of intensive research efforts and numerous clinical trials (1,3,21). A stroke is the acute neurologic injury occurring as a result of several pathological processes involving the blood vessels of the brain. Normal brain function requires continuous supply of oxygenated blood. Reduction in blood flow may interfere with brain functions, but the brain can remain viable for more prolonged periods (37), for example after a stroke patients often recover partially or completely, suggesting that focal areas of the brain can remain functionless and ischemic for hours, even days. This has led to the notion of an ischemic zone (penumbral or halo) that surrounds the infarct area and could progress up to neuronal death or is potentially salvageable if ischemia can be reversed (11,37). There are also secondary phenomena that may contribute to neuronal death such as excitatory amino acids...
(Glutamate and Aspartate) released by damaged neurons (Excitotoxicity), cerebral oedema and alterations in local blood flow owing to endothelial responses. (5,11,37) A small number of ischemic stroke patients are eligible for thrombolytic therapy with tissue plasminogen activator (t-PA) but this has to be administered within 3 hours of the ischemic event (5). However, several potential side effects have been reported.

There is much information concerning the early use of neuroprotection in ischemic stroke and brain trauma. There is a lot of evidence indicating that NMDA-receptors (N-Methyl D-Aspartate-receptors) and voltage-dependent calcium channels could be one of the triggers of neuronal injury after ischemic stroke (Xion et al, 2004) (51). Choi (1988) (7) and others (5,27) first worked with drugs that block calcium influx into ischemic cells; either conventional calcium channel blockers or Glutamate receptor blockers have had variable success in patients with stroke an in animal models. They demonstrate that the early use of NMDA-Blockers (N-Methyl D-Aspartate-Blockers) could prevent the progression of the stroke in patients who undergo cardiac catheterization. Although these results were promising, there are a lot of researchers who used these drugs with poor results (8,27,28). Several drugs that seemed promising in experimental studies or in small trials (including Glycine-NMDA-Receptor-Antagonists) have proved ineffective in phase 3 trials (8,21,28,29). The observed lack of efficacy of these drugs may be due to delays in the initiation of the treatment; however the dichotomy of NMDA-receptor signalling is a more plausible explanation (40). In pathological scenarios such as ischemia, calcium influx through the NMDA-receptor is a key mediator of cell death. Nevertheless, physiological levels of NMDA-receptor activity can promote neuronal survival and resistance to trauma, and play important roles in synaptic plasticity. (40). There is evidence that physiological synaptic NMDA-receptor activity exerts a neuroprotective effect. It may play a role in promoting recovery and preventing delayed neuronal los in the penumbra (17).

The antiepileptic drug Lamotrigine is a phenyltriazine derivative that acts by stabilizing voltage-sensitive sodium channels in a usage-dependent manner, preventing glutamate and aspartate release and reversibly blocking excitatory neurotransmission. In previous studies has been demonstrated to be effective for hypoxic-ischemic brain damage in focal and global stroke models (9,12,45,50,53) as well as combination therapy for the patient with ischemic stroke (6). Low doses of Lamotrigine could avoid CNS-adverse events and others side effects. On the other hand these doses could prevent global NMDA antagonists that may block NMDA-receptor-activated pro-survival signal triggered in response to an ischemic challenge. This dichotomy of NMDA-receptor signalling (16) means that any anti-excitotoxic strategy that interferes with NMDA-receptor signalling should be assessed to determine its effects on NMDA-receptor pro-survival signalling.

Total circulating serotonin includes platelet-serotonin (p-5HT) + free-serotonin (f-5HT) in the plasma. There is a lot of evidence that f-5-HT plasma levels augment in cerebrovascular diseases and trigger platelet aggregation enhancing the endothelium-dependent vasoconstriction, which worsens the ischemia. The fact that a small dose of oral Tianeptine, a drug that enhances serotonin uptake, reduces f-5HT in the plasma (23,24,25) motivates us to include it in the neuroprotective treatment. On the other hand we have been using Tianeptine during the last 14 years to reduce the f-5HT plasma levels in asthmatic patients with successful results. (25)

In literature it has been proved that Nitric Oxide (NO) induces vascular smooth muscle relaxation (Palmer et al, 1987) (38), and is synthesized in the endothelial cells from L-Arginine (Palmer et al, 1988) (39) by the enzyme nitric oxide synthase (NOS), and it modulates a wide variety of neural, cardiovascular, endocrinologic and humoral processes. The endothelial NO release is reduced in stroke due to endothelial factors particularly in the cerebral vasculature. (31,46). On the other hand Agmatine, formed by the decarboxylation of L-arginine by arginine decarboxylase, has been shown to be neuroprotective in trauma and ischemia models (19,36).

The current treatment for stroke relies on the use of thrombolytic agents, which are of demonstrable value only if delivered within three hours after the onset of the stroke. Although potential side effects must be considered, a neuroprotective treatment that reduces the Glutamate releases avoiding to block the pro-survival effects of NMDA-receptor activity, enhances the endothelial NO and reduces f-5HT could be an attractive option for new stroke and cerebral trauma therapies. In the present study we use low doses of Lamotrigine (Inhibitor of glutamate and aspartate release) + L-Arginine (An endothelial NO precursor) + Tianeptine (drug that reduces the f-5HT) in stroke and cerebral trauma patients to reverse the progression of cerebral ischemia toward cell death by necrosis or apoptosis.
Material and Methods

Subjects: 131 patients (78 male-53 female) between 18-85 years old conformed the eligible patients group. The institutional review board of each participating centre approved the protocol.

Inclusion Criteria

• Clinical diagnosis of stroke or cerebral trauma.
• Onset of symptoms to time administration: Patients who had suffered the stroke less than 48 hours before treatment.
  In cerebral trauma patients who did not respond to conventional therapy (Manitol, Somazina and conventional treatment) during at least 4 days.
• CT Scan showing cerebral ischemia or oedema/haemorrhage (cerebral trauma).
• Age > 30-year-old patients with stroke
• Age >18 year-old patients with cerebral trauma.
• In Stroke patients: National Institute of Health Stroke Scale (NIHSS) score 4-20

Exclusion Criteria

• Glucose > 200 mg/d
• Fever
• Hypercalcemia
• Minor stroke symptoms and TIA or NIHSS less than 4 points.
• Extensive stroke with Glasgow of 3 points and NIHSS upper 20 points.
• Haemorrhage Stroke.

Baseline clinical assessment

At baseline, details of the medical history were established by interview and consultation of medical notes. (See table 2) Patients with stroke were examined neurologically and classified as total anterior circulation syndrome (TAC), partial anterior circulation syndrome (PAC), lacunar syndrome (LAC) and posterior circulation syndrome (POC) using the Oxfordshire Community Stroke Project (OCSP) Classification (4) (See table 3). Cerebral MRI confirmed all cases within 3-7 days since the stroke occurred. Neurological deficit was scored using the National Institute for Health Stroke Scale (NIHSS). The NIHSS was included because it is the most widely used stroke scale.

The Glasgow Scale was used to evaluate the coma state in all the groups.

The Group A (Sample group)

74 patients (50 Male-24 Female) sub divided in two sub-groups:
  A.1) 49 patients with cerebral trauma (39 male-10 female-18 to 60 years old)
  A.2) 25 patients with stroke (11 male-14 female-60 to 85 years old)

All the A.1 group patients had cerebral trauma (Hospital Rafael Medina Jimenez-La Guaira-Venezuela and Hospital Domingo Luciani IVSS-Caracas-Venezuela) with traumatic haemorrhage and or cerebral oedema. Most of them with skull fractures and coma categorized as “Diffuse axonal injury”. (See table 1) The study began with those patients who did not respond to conventional therapy during at least 4 days from the entrance to the intensive care unit.

To evaluate the disease progression we used the Glasgow Scale at the beginning of the study and at the discharge of the intensive care unit. In order to maintain the same clinical conditions we compared the A.1 patients group to a 32-patients control group (20 male-12 female) with cerebral trauma who did not respond to conventional treatment after 4 days in the intensive care unit.

All the A.2 group patients (25 patients) had cerebral ischemia (Stroke) (Centro Clinico Profesional Caracas-Caracas-Venezuela and Policlinica Las Mercedes-Caracas-Venezuela). 14 male-11 female. The study began in the first 48 hours since the onset of the symptoms. To evaluate the disease progression we used the Glasgow Scale and the NIH Stroke Scale at the beginning of the study and then every 24 hours. In order to maintain the same clinical conditions we compared the A.2 group patients with a control group of 25 patients sex-age-matched (8 male-17 female), they received conventional treatment. The study began in July-2008 and was completed in December-2010.

If the f-5HT and Glutamate worsen the ischemic and neuronal injury (cerebral trauma) and enhance the progress up to neuronal death via apoptosis or necrosis, then with a treatment that enhances the eNO, reduces the f-5HT and reduces partially the activation of the NMDA-receptors, the reduction of the progression of cerebral ischemia and the changes of the clinical patient’s evolution could improve.

According to the above-mentioned statement the eligible group patients (A.1 and A.2) were treated orally with 12.5 mg of Tianeptine (drug that reduces f-5HT), 25
mg of Lamotrigine (Inhibitor of Glutamate) and 500 mg of L-Arginine (An NO precursor) twice daily during 10 days and then once daily.

**Protocol for Collecting Data**

4 different physicians carried out the experimental procedures. Two of them with the A.1 group (specialists in critical care medicine). The remaining two with the A.2 group (specialists in internal medicine).

- The Glasgow scale and the NIHSS were used in the sample group and control group with stroke.
- The Glasgow scale was used in the sample group and control group with cerebral trauma.

**Statistical procedures used**

- The data were summarized by means and percent.
- We used numerical and graphical techniques throughout the study.
- Comparisons of values between control group and treated group were analyzed by F statistical test and Student's t test with \( p \leq 0.05 \).

**Table 1. Baseline characteristics of patients with cerebral trauma based on treated Group (A1) and Control**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>A1 Group Cerebral Trauma (N= 49)</th>
<th>Control Group Cerebral Trauma (N= 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-yr</td>
<td>39,14</td>
<td>43,5</td>
</tr>
<tr>
<td>Average Age by Sex in Male (yr.)</td>
<td>39,38</td>
<td>38,9</td>
</tr>
<tr>
<td>Average Age by Sex in Female (yr.)</td>
<td>38,2</td>
<td>51,16</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>79,59</td>
<td>62,5</td>
</tr>
<tr>
<td>Female sex (%)</td>
<td>20,41</td>
<td>37,5</td>
</tr>
<tr>
<td>DSF No. (%)</td>
<td>8 (16,32)</td>
<td>5 (15,62)</td>
</tr>
<tr>
<td>BSF No. (%)</td>
<td>4 (8,16)</td>
<td>3 (9,37)</td>
</tr>
<tr>
<td>DAI No. (%)</td>
<td>9 (18,36)</td>
<td>3 (9,37)</td>
</tr>
<tr>
<td>CE No. (%)</td>
<td>31 (63,26)</td>
<td>13 (40,62)</td>
</tr>
<tr>
<td>H/H No. (%)</td>
<td>28 (57,14)</td>
<td>17 (53,12)</td>
</tr>
<tr>
<td>CSF No. (%)</td>
<td>3 (6,12)</td>
<td>1 (3,12)</td>
</tr>
</tbody>
</table>

**Table 2. Baseline characteristics of ischemic stroke patients based on treated Group (A2) and control Group.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A2 Group N = 25</th>
<th>Control Group N = 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-yr</td>
<td>74.6</td>
<td>75.2</td>
</tr>
<tr>
<td>Male sex Age-yr</td>
<td>76.9</td>
<td>75.3</td>
</tr>
<tr>
<td>Female sex Age-yr</td>
<td>72.2</td>
<td>75.17</td>
</tr>
<tr>
<td>Male sex No. (%)</td>
<td>11 (44%)</td>
<td>8 (32%)</td>
</tr>
<tr>
<td>Female sex No. (%)</td>
<td>14 (56%)</td>
<td>17 (68%)</td>
</tr>
<tr>
<td>Diabetes Mellitus No. (%)</td>
<td>11 (44%)</td>
<td>13 (52%)</td>
</tr>
<tr>
<td>Arterial Hypertension No. (%)</td>
<td>10 (40%)</td>
<td>13 (52%)</td>
</tr>
<tr>
<td>Current Smoker No. (%)</td>
<td>2 (8%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Prior cardiovascular disease No. (%)</td>
<td>3 (12%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>11 (44%)</td>
<td>9 (36%)</td>
</tr>
<tr>
<td>Prior stroke or TIA No. (%)</td>
<td>2 (8%)</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>

**Table 3. Details of the stroke after the OCSP classification and cerebral MRI**

<table>
<thead>
<tr>
<th>Details of the Stroke</th>
<th>A2 Group (Ischemic Stroke) (No. = 25)</th>
<th>Control Group (Ischemic Stroke) (No. = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TACI</td>
<td>5 (20%)</td>
<td>4 (16%)</td>
</tr>
<tr>
<td>PACI</td>
<td>6 (24%)</td>
<td>8 (32%)</td>
</tr>
<tr>
<td>LACI</td>
<td>9 (36%)</td>
<td>8 (32%)</td>
</tr>
<tr>
<td>POCI</td>
<td>4 (16%)</td>
<td>5 (20%)</td>
</tr>
<tr>
<td>Unclassified (after MRI)</td>
<td>1 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>HC</td>
<td>0</td>
<td>2 (8%)</td>
</tr>
</tbody>
</table>

**Note:** All patients showed two or more findings

**TACI = Total Anterior Circulation Infarction**

**PACI = Partial Anterior Circulation Infarction**

**LACI = Lacunar Infarction**

**POCI = Posterior Circulation Infarction**

**HC = Hemorrhagic Conversion in Ischemically Infarcted Areas**

Unclassified = The MRI Showed old frontal Infarction at the 7 days, but the OCSP Classification and the Scan at 24 hours showed TACI
Results

A.1 Group (Cerebral Trauma)

Age Average: 39.14 years old
Sex:
• Female: 20.41 %
• Male: 79.59 %

Glasgow Scale Average (Pre-Treatment): 5.33 points
Glasgow Scale Average (Post-treatment): 11.29 points

(See Fig. 1)
Improved Patients: 40/49 patients (81.63 %)
Patients with Glasgow Scale improvement but without coming out of the coma state: 9 patients (18.37 %)
Mortality: 8.16 % and Sepsis: 8.16 %. (See Fig. 2)
81.63 % of the A.1 group improved the Glasgow Scale in 5.8 points or more in two weeks. (See Fig. 1)
18.36 % of the A.1 group improved the Glasgow Scale at least in 3 points but they remained in the coma state.
The time average of Glasgow recuperation in at least 5.8 points was two weeks in 81.63 % of the patients and the time average of partial recuperation was three weeks in 18.36 % of the patients.

Control Group (Cerebral Trauma)

Age Average: 43.5 years old
Sex:
• Female: 37.5 %
• Male: 62.5 %

Glasgow scale average (pre-treatment): 5.77 points
Glasgow scale average (post-treatment): 7.75 points
The Glasgow scale average pre treatment was 5.77 points and post-treatment (until 4 weeks after) 7.75 points.
Improved Patients: 5/32 patients (15.62 %)
Patients with Glasgow Scale improvement but without coming out of the coma state: 6/32 patients (18.75 %)
Patients without Glasgow Scale improvement: 21/32 patients (65.6 %)
Mortality: 21.87 % and Sepsis: 18.75 % (See Fig 2)

A.2 Group (Cerebral Stroke)

Age Average: 74.60 years old
Sex:
• Female: 14 patients (56 %)
• Male: 11 patients (44 %)

Glasgow Scale Average (Pre-Treatment): 8.8 Points
Glasgow Scale Average (Post-Treatment): 15 Points
NIH Stroke Scale Average (Pre-treatment): 16.4 Points
NIH Stroke Scale Average (Post-treatment): 3 points
(See Fig. 4 and 5)

Time of Complete Recovery based in Glasgow scale in the A.2 group

1. Patients who started the treatment within the first 3 hours since the onset of the symptoms: 8 patients (32 %). The mean time of complete recovery was 58 hours.
2. Patients who started the treatment within the first 6-36 hours (mean time: 12.44 hours) since the onset of the symptoms: 17 patients: 68 %. The mean time of complete recovery was 5.7 days.

Time of Complete Recovery based in NIHSS in the A.2 group

1. Patients who started the treatment within the first 6 hours since the onset of the symptoms: 15 patients (60 %). The mean time of the complete recovery was 51.2 hours.
2. Patients who started the treatment within the first 8-36 hours since the onset of the symptoms: 10 patients (40 %). The mean time of complete recovery was 5.28 days.

Control Group (Cerebral Stroke)

Age Average: 75.24 years old
Sex:
• Female: 17 (68 %)
• Male: 8 (32 %)

Glasgow Scale Average Pre-Conventional Treatment: 9.48 points
Glasgow Scale Average at 72 hours: 12.4 points. (See Fig. 8)
NIH Stroke Scale Average pre-treatment: 16.84 points
NIH Stroke Scale Average post-treatment (24 hours): 16.24
NIH Stroke Scale Average post-treatment (48 hours): 15.72 points
NIH Stroke Scale Average post-treatment (one week): 12.72 points
NIH Stroke Scale Average post-treatment (two weeks): 10.12 points (See fig. 6)
Fig. 1. Comparison by sex of the Glasgow Scale at admission (GA) and at discharge (GD) in the A1 Group (cerebral trauma) and the Control Group.

In all cases $P$ value $< 0.05$

Fig. 2 Complications in cerebral trauma (Sample group and control)

Fig 3. Glasgow comparison between Treated and Control Group at admission and discharge in cerebral trauma

$P$ value $< 0.05$
NIHSS: National Institute Health Scale Score

NIHSS1: At admission; NIHSS2: At 24 hours; NIHSS3: At 48 hours; NIHSS4: At one week; NIHSS5: At two weeks.

P value < 0.05 in NIHSS2, NIHSS3, NIHSS4 and NIHSS5.

Fig. 5. Glasgow evolution in the A2 Group (Ischemic Stroke)

GO: Glasgow at admission; G12: Glasgow after 12 hours; G24: Glasgow after 24 hours; G48: Glasgow after 48 hours; G72: Glasgow after 72 hours.

P value < 0.05 in G12, G24, G48 and G72.

Fig. 6. Evolution of NIHSS in Control Group by sex (Ischemic Stroke)
Statistical analysis

We decided compared in the analysis of the A2 group the Glasgow scale at the 12, 24, and 48 hours. We compared the groups separated by sex.

The analysis of Glasgow scale on samples cases and control were relevant statistically at the 12, 24, and 48 hours, with \( p=5.41\times10^{-5} \), \( p=5.21\times10^{-13} \), \( p=3.57\times10^{-17} \), respectively.

We decided compared in the analysis of the A2 group the NIHSS at the 24 hours, 48 hours, and one week. We compared the groups separated by sex.

The analysis of NIHSS on samples cases and control were relevant statistically at the 24 hours, 48 hours, and one week with \( p=1.00\times10^{-9} \), \( p=3.04\times10^{-19} \), \( p=3.67\times10^{-27} \), respectively.

We decided compared in the analysis of the A1 Group the Glasgow Scale at discharge. We compared the groups separated by sex.

The analysis of Glasgow Scale on samples cases and control were relevant statistically \( p=2.49\times10^{-6} \). (See Fig. 9 and 10)
Fig. 10. NIHSS Comparison Between Treated and Control Group in Stroke Patients

Discussion

A new neuroprotective treatment that combines low doses of an Inhibitor of glutamate release drug (Lamotrigine) with endothelial-protection drugs (L-Arginine + Tianeptine) seems to be a promising therapeutic approach. Our results show a novel and effective neuroprotective treatment. Until now there have been no previous studies that use neuroprotective treatment with L-Arginine and Tianeptine. This ineditied study opens the door to the search of integral therapy. Our findings demonstrate that low doses of agents acting indirectly on glutamate release act better in stroke patients than usual doses of NMDA-Blockers as indicated in previous reports. (5,11,21,29) On the other hand our findings in patients with cerebral trauma confirm this assertion. To date, many cytoprotective drugs have reached the stage of pivotal phase 3 efficacy trials in acute stroke patients. Unfortunately, throughout the neuroprotective literature, the phrase "failure to demonstrate efficacy" prevails as a common thread among the many neutral or negative trials, despite the largely encouraging results encountered in preclinical studies. The reasons for this discrepancy are multiple and have been discussed (21), but recently, Papadia and Hardingham (2007) (40) explain it through the dichotomy of NMDA receptor signalling.

Failed Clinical Trials for Stroke with NMDA receptor antagonists

The calcium channels antagonist that has undergone the most extensive investigation in stroke is nimodipine (13,42). Several randomized controlled clinical studies have conclusively demonstrated the effectiveness of nimodipine in preventing ischemic neurologic deficit and poor outcome secondary to aneurismal subarachnoid hemorrhage. (42) The most recent and extensive meta-analysis of 22 calcium antagonist trials, studying over 6,800 patients, failed to demonstrate any beneficial effect of treatment, a finding attributed to hypotension induced by both oral and intravenous administration of the drug. The lack of effect or presence of detrimental effect, of calcium antagonists may be due to the hypotension caused by blocking the vascular smooth muscle cells. Another plausible explanation is that the P13K-Akt pathway (phosphoinositide-3-kinase-Akt kinase cascade) is a key-signalling pathway responsible for pro-survival effects of NMDA-receptors activity that can be activated in a calcium-dependent manner. (40,18). On the other hand, the CREB (cAMP response element-binding protein) a key mediator of activity-dependent gene expression is strongly induced by NMDA-receptor activity and calcium. (40)

Glutamate antagonists N-methyl-D-aspartate (NMDA) receptor antagonists were the first class of acute stroke therapeutic agents to proceed from development in the laboratory to testing in humans, employing modern principles of clinical trial design, most importantly relatively early treatment. The potential utility of NMDA antagonists in stroke was first recognized when it was observed that a hypoxic or ischemic insult results in elevation of brain levels of the excitatory neurotransmitter glutamate. The excitotoxic theory of ischemic brain injury implicates glutamate as a pivotal mediator of cell death via ligand-gated receptors (NMDA and AMPA receptors). The NMDA receptor is a complex ligand-gated ion channel that requires activation by glutamate and glycine, as well as concomitant membrane depolarization to overcome a voltage-dependent block by magnesium ions. Selfotel (CGS19755) is a competitive NMDA receptor antagonist that limits neuronal damage in animal stroke models (13,14). Selfotel was evaluated in a randomized, double-blind, placebo-controlled, ascending dose phase 2a study. Neuro-psychiatric adverse experiences were common, dose-related symptoms included hallucinations, agitation, confusion, dysarthria, ataxia, delirium, paranoia, and somnolence. Patients presented mild adverse experiences with Selfotel 1.5 mg/kg; however, when the dose was increased to 2 mg/kg given once or twice, adverse experiences occurred in all patients (15). The non-competitive NMDA antagonist dextrorphan was also evaluated in a pilot study (2) As with Selfotel, adverse effects of dextrorphan occurred in a dose-dependent manner. The reasons for this discrepancy
are well explained by Papadia S. and Hardingh E. (2007) (40): “NMDA-receptors are essential mediators of synaptic plasticity and also mediate aspects of development and synaptic transmission. However, when excessively activated, NMDA-receptors cause cell death in many neuropathological scenarios. During an ischemic episode, extracellular glutamate builds up due to synaptic release and impaired/reversed uptake mechanisms resulting in overactivation of NMDA-receptors. The destructive effects of excessive NMDA-receptor activity are in contrast to the recent findings that survival of several neuronal types is dependent on physiological synaptic NMDA-receptor activity (16). Thus, responses of neurons to glutamate or NMDA follow a bell-shaped curve: both too much and too little NMDA-receptor activity is potentially harmful” (30).

**Magnesium (Mg^2+)** is an ideal neuroprotectant based upon its diverse mechanisms of action, low cost, ease of administration, wide therapeutic index, good blood-brain barrier (BBB) permeability, and established safety profile. Mg^{2+} ions endogenously function as a physiologic voltage-dependent block of the NMDA receptor ion channel and inhibitor of ischemia-induced glutamate release (28). Preclinical models show that magnesium reduces infarct volume with a dose-response relationship demonstrated within even 6 hours after stroke (28,52). Several pilot studies have already demonstrated the safety and tolerability of intravenous Mg^{2+} in acute ischemic stroke patients (33,34), The FAST-MAG (Field Administration of Stroke Therapy-Magnesium) (44), pilot study was an open-label evaluation of the safety and feasibility of paramedic-initiated magnesium therapy to stroke patients identified in the field by the Los Angeles Prehospital Stroke Screen (LAPSS). Greater than two-thirds of patients had a good functional outcome. Probably the ion Magnesium showed to be more effective treatment because its action is less aggressive in blocking the NMDA-receptors, allowing the effects on NMDA-receptors pro-survival signalling.

Our study has been made to develop strategies inhibiting glutamate-induced damage while avoiding the toxicity profile of direct NMDA receptor antagonism. In fact, we used in this study low doses of Lamotrigine to prevent downregulation of neural receptors and to avoid the complete disappearances of the glutamate in the synapses, in order to allow the physiological NMDA-receptor signalling. To increase the effect of the treatment we added two unusual endothelial protection drugs (Tianeptine and L-Arginine) to prevent cell injury by other mechanisms like hypoxia and low blood flow. If we combining neuroprotective agents that together have high potency by targeting multiple pathways then the citoprotection could be effective. The anti-epileptic drug lamotrigine inhibits glutamate release and has shown beneficial effects in a rodent model of focal cerebral ischemia when administered immediately after ischemia (9,22,45); however, a 2-h delay of treatment produced no effect on infarct volume or neurological outcome in two models. (47) To our knowledge, only one clinical stroke trials of Lamotrigine have been performed but with doses that could block the physiological NMDA-receptor signalling (6).

Taken in their entirety, the data suggest that monotherapy targeting a single neurotransmitter function may not provide sufficient neuroprotection to offer clinically meaningful benefit and the doses used produced neuro-psychiatric adverse experiences that could be explained by “The dichotomy of NMDA receptor signalling”.

**The Dichotomy of NMDA receptor signalling**

The NMDA subtype of ionotropic glutamate receptors plays a Jekyll and Hyde role in the mammalian central nervous system (40). There is a lot of evidence that indicates the NMDA-receptor activated plays a dual role. If the stimulus is intense or too low then NMDA-receptor activity promotes cell death. (40) The classical bell-shaped curve model of the neuronal response to NMDA or glutamate contends that intermediate, physiological NMDA-receptor activity levels are necessary for neuroprotection. We infer that our results are explained by this novel concept. The very low doses of Lamotrigine that we used were able to reach physiological NMDA-receptor activity levels. On the other hand is possible that the Lamotrigine may have prevented the action of Glutamate and Aspartate in the extrasynaptic NMDA-receptors. A recent study involving genome-wide expression analysis has extended the understanding of synaptic vs. extrasynaptic signalling (54). While synaptic NMDA-receptors activated a number of pro-survival genes, extrasynaptic NMDA-receptors failed to do this, and activated expression of a gene Clca1 that kills neurons.

**Serotonin and Ischemia**

Serotonin plays an important role in ischemia. In humans, serotonin is concentrated in platelets and is released when platelets aggregated. The f-5HT plasma levels increase in vascular thrombosis secondary to platelet aggregation. Thus, this neurotransmitter increases the
platelet aggregation and vasoconstriction (31, 43) that worsens the ischemia. It has been demonstrated in cerebral arterioles of rabbits (31, 43) and in coronary arteries. (43). On the other hand, increased circulating catecholamines are responsible for the lowered p-5HT and the increased f-5HT registered during stressful situations, which trigger platelet aggregation. (24, 25). We used Tianeptine in this study in order to reduce the plasma levels of f-5HT owing to the fact that this drug enhances the platelet uptake of serotonin.

Recent studies show that Tianeptine targets the phosphorylation-state of glutamate receptors at the CA3 e/a synapse. This novel signal transduction mechanism for Tianeptine may provide a mechanistic resolution for its neuroprotective properties (20). On the other hand local Tianeptine has found to inhibit the activity of nitric oxide synthase (NOS) in the hippocampus (49).

**Nitric Oxide and Agmatine**

Other strategies of neuroprotection attack later stages of the ischemic cascade. Neuronal nitric oxide (nNO) synthesis is induced by stimulation of glutamate receptors, and nNO in turn has a number of complex actions relevant to ischemia and cell injury. Endothelium-derived NO (eNO) causes vasodilatation beneficial to ischemic brain, but nNO generates oxygen free radicals toxic to cells. The usefulness of NO modulation in stroke likely will hinge on the ability to favourably manipulate the beneficial and deleterious effects of NO. We decided to use low doses of L-Arginine like a precursor of NO due to the important role that endothelial NO plays in the cerebral vasodilatation, vascular remodelling and angiogenesis in human and animal models (21,26). In our opinion low doses of L-Arginine increase NO at low levels that are not toxic to cells. On the other hand, L-Arginine blocks the release of somatostatine thus increases the Grow Hormone levels that have anti stress and cell-reparative properties (26). Moreover, L-Arginine is a precursor of the novel neuroprotective Agmatine that has been shown to be neuroprotective in trauma and ischemia models through regulation of endothelial nitric oxide synthase, reduction of brain edema and glutamate release (19,36). On the other hand, agmatine can reduce brain infarction through minimizing neuroinflammation and can lessen the danger of post-stroke infection from depression of the immune system after stroke (48).

Free radicals production occurs during ischemia and reperfusion and contributes to the neuronal injury after stroke. In order to avoid the effect of NO as a free radical we reduced the doses of L-Arginine after two weeks of treatment.

Our study has potential limitations. One of them is the imbalance in the two sample groups. The A1 group included cerebral trauma patients with ostensible cerebral damage. The A2 group included only cerebral stroke patients with only ischemic damage. In the former the most important evaluation was the use of low doses of lamotrigine to avoid the progression of the cerebral injury (Low doses owing to the fact that the experience in neuroprotection studies with NMDA blockers shows many side effects). In the latter the key was the use of three different mechanisms to avoid the progression and damage of the penumbra zone. Other limitation was the fact that the A1 group began the treatment 4 or more days after the cerebral trauma occurred, and this group included only patients catalogued as “Did not respond to the usual therapy”. There is a disadvantage because there is a lot of information concerning the beneficial use of early neuroprotective treatment in phase I and II studies. Other limitation is that we began the treatment in the A2 group earlier and we cannot compare it to the A1 group.

Our results show that the use of Lamotrigine + L-Arginine + Tianeptine in cerebral trauma and ischemic stroke results in higher survival rates and lower rates of neurologic complications at one week since the onset of the symptoms. On the other hand, this treatment significantly reduces the clinical-recuperation-time of Glasgow scale and NIH stroke scale when it was compared with the conventional treatment.

Since the Food and Drug Administration has recognized that stroke is a serious and life-threatening condition, making it eligible for accelerated approval, we believe that this treatment can be extrapolated to institutions with resources in stroke and cerebral trauma trial. An inexpensive, effective and safe neuroprotective treatment could be evaluated in a large-scale, multicenter, double-blind, placebo-controlled, randomized trials.

Finally, we believe that delineating the mechanism underlying the vulnerability of the central nervous system to diverse insults should lead to new therapeutic interventions that affect the outcome positively.

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