THE EFFECT OF ASCORBIC ACID ON GLUTAMATE INDUCED BEHAVIORAL CHANGES

SHAHIDA P. AHMED, RAFEEQ ALAM KHAN MAHAYROOKH AND SAIDA HAIDER*

Department of Pharmacology, Faculty of Pharmacy, University of Karachi
*Department of Biochemistry, University of Karachi
Karachi-75270, Pakistan

ABSTRACT:
The cellular and molecular factors responsible for neurotoxic effects (e.g.) tardive dyskinesia are incompletely understood. Accumulation of free radical species (ROS) is major contributing factor for such effects. The observation that suppression of glutamate decarboxylase in substantial nigra and increase release of glutamic acid support that neurotoxic effects of glutamic acid mediate through NMDA receptor and could result in excitotoxic effects. Present study reveals locomotion disorder and impaired cognition in animal models. These effects are possibly occurring due to release of glutamate and accumulation of ROS. In present work vitamin C was effective in some of the animal models of excitotoxicity with impaired cognition and movement disorder.

INTRODUCTION

In recent years there has been exponential growth of basic clinical research directed towards brain’s own Excitatory Amino Acid (EAA) neurotransmitter system and excessive acute or chronic derangements of neuronal metabolism. These include increased intracellular calcium, increased free radical formation and defect in energy metabolism of brain. Thus breaking down the cellular defenses leading to lipid peroxidation, neuronal injury and death. Excitatory amino acids (EAA) are most likely to be a causative factor for neurological diseases. Chronic diseases forms may be Huntington’s Chorea Alziemer’s disease and Creutzfelt Jakob Syndrome (Griffin and Price, 1980 and Griffin et al., 1984).

Glutamate is involved in peptide and protein synthesis also in detoxification of ammonia in brain. Precursor of GABA is also glutamate. With regard to the possible role of glutamate in psychiatric and neurological diseases, there is growing evidence that glutamate is involved in brain damage may be resulting from cerebral anoxia in case of stroke or possibly in epilepsy. In present study the effect of Ascorbic acid is evaluated. Ascorbic acid is antioxidant vitamin, preventing free radical formation. Present study is planned to evaluate the preventive effect of vitamin C.

Glutamate and certain other acidic amino acids are excitatory neurotransmitters within the central nervous system (CNS). The discovery that these excitatory amino acids are neurotoxic at a certain concentration in the brain has generated a great amount of interest in these “excitotoxins”. In vitro systems have established that the toxicity of glutamate can be blocked by certain glutamate antagonists (Rothman and Onley, 1986), and the concept is rapidly emerging that the toxicity of excitatory amino acids may be related to such divergent conditions as hypoxia, epilepsy and neurodegenerate diseases (Meldrum, 1987 and Choi, 1988).

Glutamate and aspartate are the most abundant amino acids in mammalian brain.
The importance of glutamate as excitatory amino acid (EAA) is well recognized. Despite the many roles it play in intermediary metabolism and transmitter function, studies on the dentate gyrus of the hippocampal formation, where glutamate has been established as a transmitter have shown that the synthesis of glutamate is regulated by glutamine. Present work is designed to evaluate the effect of Ascorbic acid on behavioral changes induced by administration of glutamate acid (McGeer et al., 1978).

MATERIALS AND METHODS

Male Albino mice (25-32g) were used. Animals were kept at room temperature with 12 hr light and dark cycle and had free access to food and water. Experiments were always conducted between 3pm – 6.pm.

Drugs used for the treatment were:

a) Glutamic acid (GLU) available in amorphous form (sigma chemicals).

b) L-Ascorbic acid available in tablet form (100mg Abbott Laboratories).

Experimental Dose:

a) Experimental dose of glutamic acid (GLU) =5 µ mol/litre.

b) L-Ascorbic acid (Vitamin C). 04mg.

c) Duration of administration was one month.

Mice were randomly assigned as controls, group A and group B. In each group there were six animals. To group A animals glutamic acid was given chronically for a period of six (6) weeks. Neurological and behavioral changes were noted. The group B animals were chronically co-administered both glutamic acid and L-Ascorbic for six (6) weeks Neurological and behavioral changes were noted.

Group-A animals were chronically given glutamic acid in a dose of 5mmol/litre through oral route of drug administration.

Group-B animals were chronically co-administered glutamic acid and L-Ascorbic acid through oral route of drug administration, control animals were maintained on saline.

Behavioral Parameters:

Following behavioral parameters were observed.

Measurement of Body weight:

Mice were weighed before starting the experiment, weight changes were monitored daily during the treatment.

Measurement of Food Intake:

A weighed amount of food was placed in the hopper of the cage of each animal. Food intakes were monitored daily by weighing the remaining food in the hopper.

Measurement of Water Intake:

A measured amount of water was placed in the bottle of the cage of each animal. Water intakes were monitored daily by weighing the remaining water in the bottle.

Behavioural studies:

Following parameters were observed.

1. Cage crossing activity test
2. Exploratory activity test
3. Gross behavioral analysis
4. Balance on stationary rod test
5. Traction test
6. Conditioned avoidance response (CAR)

Cage Cross Activity Test:

Cage crossing activity was noted visually for a period of 15 minutes for each mice individually for group A and B.

Exploratory Activity Test:

Exploratory activity of mice were noted with the help of head dip box for a period of 15 minutes for each mice individually.

Gross Behavioral Test:

An acute toxicity screen tests were performed. Mice were daily observed for gross behavioral changes such as awareness, mood, motor activity, CNS excitation, posture, motor incoordination, muscle tone, reflexes and autonomic reflexes. A daily gross behavioral activity tests were noted.
Balance on Stationary Rod Test:
In this test mice were allowed to balance an stationary rod after training. Time during which mice maintain balanced and then fall from the stationary rod was noted for each mice individually.

Traction Test:
In this test mice were allowed to hold a wire grid. Time during which mice were hanging and then fall off the wire was noted for each mice individually.

Conditioned Avoidance Response Test (CAR):
Rats were used to perform conditioned avoidance response lest (CAR). In this test an outer stimuli such as foot shock was given repeatedly. The response was noted for each rat individually.

Statistical Analysis:
Behavioral data were statistically analyzed by student T-test. P<0.05 was considered significant.

RESULTS

Glutamic acid (GLU) is an excitatory amino acid (EEA). Excitatory amino acid may produce central nervous system hyper stimulation and neuronal loss. Present work was conducted to observe if there is any adverse effect induced by glutamic acid and prevention by natural antioxidant i.e., L-ascorbic acid.

Mice were assigned as control, Group-A and Group-B. in each group there were six (6) mice. To Group-A glumatic acid and to Group-B glutamic acid with L-ascorbic acid was co-administered for a period of six (6) weeks.

Group-A (Results):

1) Cage Cross Activity Test:
The results are shown in Table-1 and Fig.3.1. The data analyzed by T-test showed a significant decreased on total counts of cage cross (mean=48.24S, T value=3.467, P<0.05).

2) Exploratory Activity Test:
The results are shown in Table-2 and Fig.3.2. The data analyzed by T-test showed a significant decreased on total counts of head dip (mean=40.82, T value=3.152, P<0.05). The results shows that Group-A animals that were chronically given glutamic acid results in highly diminished exploratory activity as compare to control animals. Test was performed on mice.

Gross Behavioral Activity Test:
Gross behavioral activity was observed. It was noted that alertness was negative. Visual placing was normal. Passivity is significant and no stereotypy behavior was observed.

No significant effect on grooming. Restlessness was positive. No, vocalization was noted. Mousses were irritable and aggressive showed fighting behavior. Motor activity was slow. Animals were dyskinetic. After chronic administration of glutamic acid reactivity was negligible. Touch response was positive. Pain response was positive.

Startle response was absent. Alopecia was highly significant. Limb position was affected. Staggering gait was noted. There was negative righting reflex. Grip strength was positive. Body sagging was negligible. Pupil size was normal. No exophthalmous. Excessive salivation. There was slight skin discoloration. Respiration apparently seems to be normal. Death of one mice occur due to aggressive fighting behaviour.

4) Balance on Stationary Rod Test:
The results are shown in Table-3 and Fig.3.3. The data analyzed by T-test showed a significant decreased on total time of balance on stationary rod The results shows that Group-A animals that were chronically given glutamic could not maintain balance on stationary rod as compare to control animals.
The Effect of Ascorbic Acid on Glutamate

5) Traction Test:
Mice fall off from wire readily as compare to control animals.

6) Conditioned Avoidance Response Test:
**(CAR)**
Rats are used for this test. After chronic co-administration of glutamic acid rats avoided signals (foot’ shock) repeatedly.

**Group-B (Results)**
1) Cage Cross Activity Test:
The results are shown in Table-1 and Fig.3.1 The data analyzed by T-test showed a significant decreased on total counts of cage cross. The results show that animals that were chronically co-administered glutamic acid and L-ascorbic acid results in highly diminished cross cage activity as compare to control animal’s and it has no significant improvement in cross cage test as compare to Group-A animals.

2) Exploratory Activity Test:
The results are shown in Table-2 and Fig.3.2. The data analyzed by T-test showed a significant decreased on total counts of head dip. The results shows that Group-B animals

---

**Table-1**
Showing values of cage crossing

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group of Mice</th>
<th>Drugs</th>
<th>Dose</th>
<th>Mean ± S.E.M. (n=36)</th>
<th>S.D.</th>
<th>T-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control Mice</td>
<td>-</td>
<td>-</td>
<td>53.110 ± 0.410</td>
<td>2.44</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Group-A</td>
<td>Glutamic Acid</td>
<td>5mml/liter</td>
<td>48.245 ± 1.25</td>
<td>7.595</td>
<td>3.467</td>
<td>0.001</td>
</tr>
<tr>
<td>3.</td>
<td>Group-B</td>
<td>Glutamic Acid + L-Ascorbic Acid</td>
<td>20ml</td>
<td>49.180 ± 1.4</td>
<td>7.875</td>
<td>2.737</td>
<td>0.015</td>
</tr>
</tbody>
</table>

S.E.M.: Standard Error of Mean
S.D.: Standard Deviation

Fig.1: Comparative bar graph of mean of control, Group-A and Group-B (Cross-Cage Test of Mice)
that were chronically en-administered glutamic acid and U-ascorbic acid results in highly diminished exploratory activity as compare to control animals and it has no significant improvement in head dip counts as compare to Group-A animals.

3) Gross Behavioral Activity Test:
Gross behavioral activity was observed in Group-B animals that were chronically co-administered glutamic acid (GLU) and L-ascorbic acid. No significant prevention was offered by L-ascorbic acid in toxic symptoms induced by glutamic acid (GLU). It was noted that alertness was negative. Visual placing was normal. Passivity was slightly reduced. There was slightly positive effect on righting reflex. Stereotypy behavior was not obvious. No vocalization as Group-A. restlessness and irritability was suppressed significantly. No startle or fearful response was seen. Touch and pain response were positive. No appearance of straub tail. Slight convulsions were significantly reduced by L-ascorbic acid. Twitches were minimized. Body posture was abnormal after giving glutamic acid and it is not prevented by L-ascorbic acid. Hind paw abduction was prominent. Staggering gait was

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group of Mice</th>
<th>Drugs</th>
<th>Dose</th>
<th>Mean ± S.E.M. (n=36)</th>
<th>S.D.</th>
<th>T- value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control Mice</td>
<td>-</td>
<td>-</td>
<td>44.86 ± 4.20</td>
<td>2.51</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Group-A</td>
<td>Glutamic Acid</td>
<td>0.02ml of 5mm/liter</td>
<td>40.825 ± 1.2</td>
<td>7.275</td>
<td>3.467</td>
<td>0.007</td>
</tr>
<tr>
<td>3.</td>
<td>Group-B</td>
<td>Glutamic Acid + L-Ascorbic Acid</td>
<td>0.02ml of 5mm/liter 100mg/Kg</td>
<td>41.437 ± 1.225</td>
<td>7.225</td>
<td>2.717</td>
<td>0.016</td>
</tr>
</tbody>
</table>

S.E.M.: Standard Error of Mean
S.D.: Standard Deviation

Table-2
Showing data of exploratory activity test

Fig.2: Comparative bar graph of mean of control, Group-A and Group-B (Exploratory Activity Test of Mice)
The Effect of Ascorbic Acid on Glutamate

abnormal. Limb tone was slightly improved. Grip strength was present. Body sag was slightly improved by L-ascorbic acid. Pupil size was unaffected. Salivation was not significantly affected by L-ascorbic acid. Skin discoloration was slightly improved. Alopecia was improved.

4) Balance on Stationary Rod Test:
The results are shown in Table-3 and Fig.3.3. The data analyzed by 2 sample T-test showed a significant decrease on total time of balance on stationary rod (mean=1

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group of Mice</th>
<th>Drugs</th>
<th>Dose</th>
<th>Mean ± S.E.M. (n=36)</th>
<th>S.D.</th>
<th>T-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control Mice</td>
<td>-</td>
<td>-</td>
<td>31.94 ± 0.81</td>
<td>4.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Group-A</td>
<td>Glutamic Acid</td>
<td>25mg/Kg</td>
<td>8.970 ± 1.2</td>
<td>7.01</td>
<td>16.14</td>
<td>16.14</td>
</tr>
<tr>
<td>3.</td>
<td>Group-B</td>
<td>Glutamic Acid + L-Ascorbic Acid</td>
<td>25mg/Kg 100mg/Kg</td>
<td>11.36 ± 1.3</td>
<td>7.82</td>
<td>13.4</td>
<td>13.4</td>
</tr>
</tbody>
</table>

S.E.M.: Standard Error of Mean
S.D.: Standard Deviation

Fig.3: Comparative bar graph of mean of control, Group-A and Group-B (Exploratory Activity Test of Mice)

1.360 seconds, T value = 13.4, P<0.05). The results shows that Group-B animals that were chronically co-administered glutamic acid and L-ascorbic acid did not maintain balance on stationary rod as compare to control animals but there was significant improvement as compare to Group-A animals.

5) Traction Test:
Grip strength was normal. There was no significant effect on muscle tone as observed by traction test.
6) **Conditioned Avoidance Response Test: (CAR)**

Rats are used for this rest. After chronic co-administration of glutamic acid and L-ascorbic acid rats avoided signals (foot’ shock) repeatedly and there is no significant improvement in conditioned avoidance response (CAR) as compare to Group-A animals.

**DISCUSSION**

Glutamate neurotransmission is medicated by at least three subtypes of receptors that may be recognized by their specificity for kainite, quisquulate and N-methyl-D-aspartate (NMDA). The entry of glutamate into the central nervous system (CNS) is regulated at the blood brain barrier (BBB), and following an injection of a large dose of glutamate in infant rodents, glutamate exerts its effects in the area of the brain in which the blood brain barrier (BBB) is least developed, the circumventricular organ. Within this site of limited access glutamate injures neuron, apparently by opening glutamate-dependant ion channels, ultimately leading to neuronal swelling and neuronal cell death (Olney, 1978 and Coyle, 1987). The toxicity affects dendrites and neuronal cell bodies, but seems to spare axons. The only known related human condition is the “Chinese restaurant syndrome” in which consumption of large amounts of monosodium glutamate (MSG) as a seasoning may lead to a burning sensation in the face, neck and chest.

Glutamic acid (GLU) toxicity was observed significantly in most of experimental models. But L-ascorbic acid (Vitamin C) was unable in most experimental model to improve significantly glutamic acid (GLU) toxicity (Table 1-3) (Fig. 1-3) Alopecia and skin discoloration were significantly improved that is probably due to effect of L-ascorboic acid (Vitamin C) on skin. L-ascorbic acid enhanced absorption of iron that is improving texture of skin and due to its antioxidant property. Improvement in patchts is due to inhibition or prevention of peroxidation of unsaturated fatty acid. Twitches were slightly reduced after chronic administration of L-ascorhic acid indicating that some neuronal injuries induce by glutamic acid can be protected by L-ascorbic acid. Glutamic acid (GLU) in certain concentration produce neuronal loss. One of the reason of neurodegeneration is enhanced by per-oxidation and free radicals formation that may results in neurodegenerative disease and hypoxic encephalopathy.

The growing field of the exitotoxic amino acids embodies many of the same attributes that characterize the more general discipline of neurotoxicology. Neurotoxicology is generally viewed as the study of compounds that are deleterious to the nervous system, and from this mold emerges the effects of glutamate and kainite. Exposure to these excitotoxic amino acids leads to neuronal injury, and when of sufficient degree, may kill neurons. However, the implications of these findings, as with the entire field of neurotoxicology extend beyond the direct toxicity of the compounds in exposed populations. With kainite, as with many other neurotoxic compounds, has come a tool for the neurobiologist who seek to explore the anatomy and function of the nervous system. Kainite, through its selective action on neuronal cell bodies, has provided a greater understandingof the function of the cell within a specific region of the brain while previous lesioning techniques addressed only regional functionalities. Finally, the question surrounding BMAA, the cycad, aluminum, and Gusantian ALS/PD serve to remind the student of neurotoxicology that the cause of many neurologic disease remain unknown. This void in understanding, and the implication that some neurodegenerative diseases may have toxic etiologies, provide a heightened desire to appreciate more fully the effects of elements of our environment on the nervous system (Hochman et al., 1988 and Darvasi 1998).

Some patients who seem to recover from anoxic coma undergo a delayed deterioration in their neurologic state. Delayed postanoxic encephalopathy is pre-dominantly a disorder
of the white matter and is characterized by widespread demyelination of the cerebral hemispheres. When cerebral neurons are deprived of oxygen, there is a remarkable increase of extra cellular excitatory neurotransmitter like glutamate and aspartate, which open calcium channels and leads to a massive calcium influx into the neuron, this unregulated increase in calcium leads to the activation of intracellular enzymes and the consumption of adenosine 5'-triphosphate. The neuron begin to catabolize themselves to maintain activity and in so doing are damaged to a degree that does not permit their survival (Hochman et al., 1998).

A cognitive enhancer is a neuroactive substances that elevates an individual cognitive abilities in a meaningful and sustained say, beyond that individual’s basal level of performance. Another term that is sometimes used, especially in Europe, is nootropic drug. In every day life normal people seek drugs, herbal remedies, or nutritional supplements to improve their memory, ability to concentrate, or clarity of thought. People concerned with the mild memory impairment occurs in “Benign senescence” are specially likely to seek out such treatments. Pana gingseng and Gingko biloba are two popular remedies, so called nutraceuticals cognitive enhancer for normal people at least to a degree, but they are only occasionally helpful to patients who are mentally retarded or autistic. The cholinergics, on the other hand do not improve memory performance in normal subjects but may have a role for some mentally retarded patients (Nunn et al., 1987).

A neuroprotective substance is a drug nutraceutical, or dietary supplement that protect nerve cell from damage, either from pathologic events such as the excitotoxicity that accompanies stroke or brain injury or from normal biologic stressors, e.g. the generation of oxygen free radicals. There are many substances with proven neuroprotectant ability, at least in vitro. Deprenyl, for example, is said to retard progression of Parkinson’s disease (PD). Vitamin C and Vitamin E are protective against oxidant stress. Patients with brain injuries or developmental handicaps are obvious candidates for cognitive enhancement. They are good candidates for neuroprotection because they are more vulnerable to mental deterioration and dementia (Darvasi, 1998).

These results suggest that mechanism of action inducing toxicity is not only mediating through excitotoxin. Glutamic acid (GLU) through formation of free radicals and peroxidation produce neurodegenerative diseases, some other mechanisms are also involved it may be hypoxia, decreased energy metabolism and insufficient production of ATP. It is suggested present work can further be extended.

1. Present work needs intensive studies on mechanism of action of glutamic acid (CLU) induced toxicity.
2. NMDA receptor antagonist may be applied to further strengthen the results.
3. Receptor pharmacology studies are required using specific glutamate agonist and antagonist.
4. Similar type of studies may be carried out on other animal species and this would provide a comprehensive data for comparison of species differences.
5. Similar type of study may be carried out in Parkinson’s disease animals models and in clinical studies to establish the protective role of natural antioxidant i.e. L-ascorbic acid (Vitamin C).

CONCLUSION

In conclusion, the results of this study indicate that the adverse effect induced by glutamic acid (GLU) were observed significantly in most of experimental models of mice. These results also tend to suggest that mechanism of action inducing toxicity is not mediating through excitotoxin. Glutamic acid,
through formation of free radicals and peroxidation may produce neurodegenerative diseases and hypoxic encephalopathy, some other mechanisms are also involved it may be hypoxia, decreased energy metabolism and insufficient production of ATP. These results also suggest that natural antioxidant i.e. L-ascorbic acid (Vitamin C), failed to significantly improve glutamic acid toxicity in most of experimental models; Alopecia and skin discoloration were significantly improved i.e. improvement in texture of skin is due to its antioxidant property improvement in patches is due to inhibition or prevention of peroxidation of unsaturated fatty acid. Twitches were slightly reduced, indicating that some neuronal injuries induced by glutamic acid can be protect by L-ascorbic acid.

REFERENCES


