

Role of Ketamine, Levetiracetam and L-Carnitine in Aminophylline Induced Seizure in Wister Rat Model

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ABSTRACT

Background: Aminophylline-induced seizure is commonly fatal and no specific, pharmacologically antagonistic drug is known. But recently, free radicals have been implicated in Aminophylline induced toxicities.

Aim: The present study was conducted whether simply anticonvulsant drug or anticonvulsants that mediated by reduction of oxidative stress or just antioxidant can alleviate the aminophylline-induced seizure threshold as well as mortality.

Method: Male Wister rats (n = 40) were randomly selected and divided into four groups. One group was injected Aminophylline 300mg/kg intraperitoneally (control group), second group was administered aminophylline 300 mg/kg intraperitoneally 5min after giving Ketamine (100mg/kg i.p.), in third group l-carnitine, was administered intraperitoneally with a rapid infusion (0.05 g/kg) diluted in 250 ml of saline solution before introducing Aminophylline 300mg/kg intraperitoneally and third group Levetiracetam (200 mg/kg, i.p.) was injected intraperitoneally 60 min before inducing Aminophylline-induced seizures (300 mg/kg, i.p.).

Result: Pretreatment with Ketamine, showed no antagonizing effects on seizurogenic effect of aminophylline (300 mg/kg) and 24 hr post-seizure mortality. But when L-carnitine and Levetiracetam were given isolated therapy before the aminophylline they reduce 10% and 40% aminophylline induced seizure threshold as well as mortality respectively. Malondialdehyde and Protein carbonyls product formation were significantly suppressed and Superoxide dismutase activity was improved in different areas of brain by L-carnitine and Levetiracetam administration ($p < 0.001$) than control group whereas Ketamine neither decreased oxidative stress nor seizure threshold as well as mortality significantly.

Conclusion: Anticonvulsive drug that are acted by correction of disturbed redox balance is better to treat Aminophylline-induced

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seizure than Anticonvulsants are acted by other ways.

Keywords: Aminophylline, Seizure, Male wister rats, Anticonvulsive drug, Ketamine, Malondialdehyde, Levetiracetam.

INTRODUCTION

Aminophylline is widely used in the treatment of bronchial asthma and chronic obstructive pulmonary disease.¹ However, due to its narrow therapeutic index it has a high toxicity potential.² A serious adverse effect associated with intravenous aminophylline therapy is seizure. Such repetitive, generalized convulsion is usually not preceded by any prior milder and warning symptoms and is relatively refractory to conventional anticonvulsant agents.³ These seizures are commonly fatal and no specific antagonistic drug is known.⁴

Ketamine is a drug used in human and veterinary medicine, primarily for the induction and maintenance of general anesthesia, usually in combination with a sedative. Ketamine offers an additional advantage in the treatment of refractory status epilepticus in that it is potentially neuroprotective.

Levetiracetam (LEV) is a new antiepileptic agent with broad-spectrum effects on seizures and animal models of epilepsy.⁵ It was also demonstrated that this effects of LEV can be mediated, at least in part, by reduction of lipid peroxidation and hippocampal oxidative stress.⁵

Carnitine is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine.⁶ The biologically active enantiomer L-carnitines exert a substantial antioxidant action, thereby providing a protective effect against lipid peroxidation of phospholipid membranes and against oxidative stress induced at the myocardial and endothelial cell level.^{7,8}

In view of the above, the present experimental study was designed to

investigate the ability of Ketamine, L-carnitine and Levetiracetam in alleviation of aminophylline-induced seizures in rat model.

MATERIAL AND METHODS

Study area

The present study was an animal model based case control study performed in the Departments of Biochemistry with the collaboration of the Department of Pharmacology of Burdwan Medical College, Burdwan, West Bengal, India.

Animal

Male Wister albino rats (*Rattus norvegicus albinus*), aged between 1 to 2 months weighing 150 ± 12 g, n = 40 were obtained for present study from the appropriately maintained institutional animal house. The rats had free access to drinking water and rat food pellets. The light source in the animal room was regulated with 12 hr light period followed by 12 h dark schedule within a temperature of range of $22 \pm 2^\circ\text{C}$ at a relative humidity of 45 to 50 %. All rats were acclimatized for at least 7 days before starting the study. All procedures involving animals were undertaken according to the 'Guide for the Care and Use of Laboratory Animals (1985), NIH, Bethesda' and 'Guidelines for care and use of animals in scientific research' by the Indian National Science Academy (INSA), New Delhi, India. The study was approved and permitted by the institutional ethics committee for care and use of laboratory animals, and the study was started after

obtaining the written consent from the concerned ethics committee [Memo No. BMC/2179/1 (10)].

Study design

The rats were randomly divided into 4 groups.

Group I (n=10)

Experiments were carried out with single doses of aminophylline 300 mg/kg administered intraperitoneally to rats and subsequently put into individual perspex cages (25×25×10 cm).

Group II (n=10)

Ketamine, 100mg/kg was made intraperitoneally to rats. As Ketamine, 100mg/kg, produced maximal behavioural impairment 5min after intraperitoneal injection,⁹ after 5 min, a single dose of aminophylline 300 mg/kg administered intraperitoneally and subsequently put into individual perspex cages (25×25×10 cm).

Group III (n=10)

l-carnitine, was administered intraperitoneally with a rapid infusion (0.05 g/kg) diluted in 250 ml of saline solution to rats. Then a single dose of aminophylline 300 mg/kg administered intraperitoneally and subsequently put into individual perspex cages (25×25×10 cm).

Group IV (n=10)

LEV (200 mg/kg, i.p.) was injected intraperitoneally 60 min before Aminophylline-induced seizures (300 mg/kg, i.p.) and subsequently put into individual perspex cages (25×25×10 cm).

Then all the rats were then observed for 60 min (1 hr), and studied – (a) Percent incidence of seizures (animals showing at least clonic seizures of forelimbs were taken for calculating % incidence of seizures); and (b) Per cent mortality within 20 min and 1

hr. All experiments were carried out in a quiet room between 10 AM and 3 PM (with constant light condition). Then all the rats in this study then were sacrificed by cervical dislocation. (See figure 1.)

Tissue sample preparation

To determine oxidative changes caused by free radicals in different area of brain, brains of the following four group rats were dissected and kept in the following order cortex, cerebellum, midbrain and the basal ganglia. The brain tissues were mildly washed in normal saline to remove the blood and after this were frozen immediately, at first at –20 °C and then at –70 °C, and kept under these conditions (–70 °C) until chemical analysis was performed. For homogenisation, samples were first washed and then minced with sharp surgical blade in small volumes of ice cold (not frozen) homogenisation buffer made of 0.1 M Tris-HCl (pH 7.35) and 100 μM ethylenediaminetetraacetic acid (EDTA). Immediately the samples were homogenised in 10 volumes of the ice cool buffer solution in a motor driven glass tissue homogenizer in presence of properly washed few particles of sand. During the whole homogenisation procedure the homogeniser was kept submerged in small ice particles to dissipate any heat. Thereafter the samples were centrifuged at 10,000 rpm for 10 min in a refrigerated cold centrifuge machine at 4°C. Supernatants from the homogenates were collected and MDA, PC adducts, cytosolic superoxide dismutase (Cu²⁺-Zn²⁺-SOD) activity and tissue protein were estimated immediately in these supernatant.¹⁰

Biochemical assay

Lipid peroxidation maker MDA was measured by reacting with thiobarbituric acid at 532 nm. The level of MDA in the brain tissues were calculated using a calibration curve derived from 1, 1, 3, 3-tetraethoxypropane as the external calibration

standard. The calibration curve was linear in range from 1.25 to 2.5 nmol/ml ($r^2=0.997$).¹¹ Free radical induced oxidative changes in the tissue proteins were measured by estimating the protein carbonyl (PC) products. The method is based on the reaction of carbonyl groups of protein with 2, 4-dinitrophenylhydrazine to form a 2, 4-dinitrophenylhydrazone reactive carbonyl derivative that was measured at 370 nm.¹² Estimation of cytosolic SOD activity was done by using the method of Kakkar *et al.* where one unit of SOD was defined as that amount of enzyme that inhibited the rate of electron transfer from reduced Nicotinamide adenine dinucleotide (NADH) to nitroblue tetrazolium (NBT) by 50 % under specified conditions.¹³ Tissue proteins were measured by the Lowry method¹⁴ that involved reaction of proteins in tissue homogenates with alkaline copper sulphate followed by another reaction with Folin's phenol reagent against a standard curve prepared from bovine serum albumin. All photometric measurements were performed in Dual beam spectrophotometer (UV 5704SS). The estimated biochemical parameters were expressed in their corresponding units per mg of tissue protein.

Chemicals

1, 1, 3, 3- tetraethoxypropane was from Fluka, Germany and Folin's phenol reagent was from SRL, India and bovine serum albumin was from Merck, Germany.

Statistical analysis

The data for this study was subjected to standard statistical analysis using the Statistical Package for Social Science (SPSS) 11.5 software for windows. For all tests, the p-value was considered to be significant if it was less than 0.05 at a confidence level of 95 %.

RESULT

Effects of Ketamine, L-carnitine and Levetiracetam on aminophylline-induced seizures in Wister rats – Fisher's exact test

Pretreatment with Ketamine, showed no dose related antagonizing effects on seizurogenic effect of aminophylline (300 mg/kg) and 24 hr post-seizure mortality.

But when L-carnitine and Levetiracetam were given isolated therapy before the aminophylline they reduce 20% and 10% aminophylline induced seizure threshold as well as mortality respectively as shown in the Table1.

Differences between the mean values of oxidative stress parameters in different area of brain of different groups of rats-ANOVA with Bonferroni correction

As main aetiopathological factor of Aminophylline induced seizure is disbalance of redox status, we compare the efficiency in reduction of oxidative stress by Ketamine, L-carnitine and Levetiracetam and independent sample t test was performed between Group I rats with Group II Group III and Group IV (Table 2), it was observed that MDA and PC product formation were significantly suppressed and SOD activity was improved in different areas of brain by L-carnitine and Levetiracetam administration ($p < 0.05$, Figure 2) in Group III and Group IV rats than Aminophylline group, whereas Ketamine fail to decreased oxidative stress significantly (Figure 2). That means there was significant decrease of oxidative parameters in L-carnitine and Levetiracetam treated group III and group IV than Ketamine administered group II experimental rats.

DISCUSSION

The use of aminophylline for therapeutic purpose is associated with intractable fatal seizures and this tonic-clonic seizures is a dose-dependent pheno-

menonas.^{15,16} The mechanisms involved in such seizures are still not clearly understood and thus, the treatment of this potentially life threatening condition is not satisfactory. For these repetitive, generalized seizures no specific pharmacologically antagonistic drug is known.¹⁷ Moreover, recently it has been proposed that free radicals have a major role in development of Aminophylline induced toxicities.¹⁸ So, the present study was conducted whether simply anticonvulsant drug or anticonvulsants that mediated by reduction of oxidative stress or just antioxidant can alleviate the aminophylline-induced seizure threshold as well as mortality.

It was found that L-carnitine and more efficiently Levetiracetam can antagonise the seizure, whereas Ketamine fail to do this. It was also observed that MDA and PC product formation were significantly suppressed and SOD activity was improved in different areas of brain by L-carnitine and Levetiracetam administration than Aminophylline group, whereas Ketamine fail to decrease oxidative stress significantly. That means there was significant decrease of oxidative parameters in L-carnitine and Levetiracetam treated group III and group IV than Ketamine administered group II experimental rats. This effect is brought out by correction of disturbed redox balance by these two drugs. In contrast, ketamine has no role on oxidative stress so it fails to correct the seizure threshold as well as mortality rate.

CONCLUSION

The results are suggestive that anticonvulsive drug that are acted by correction of disturbed redox balance is better to treat Aminophylline-induced seizure.

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Conflict of interest

We do not have any conflict of interest.

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Table 1. Effects of Ketamine, L-carnitine and Levetiracetam on aminophylline-induced seizures in different groups of rats

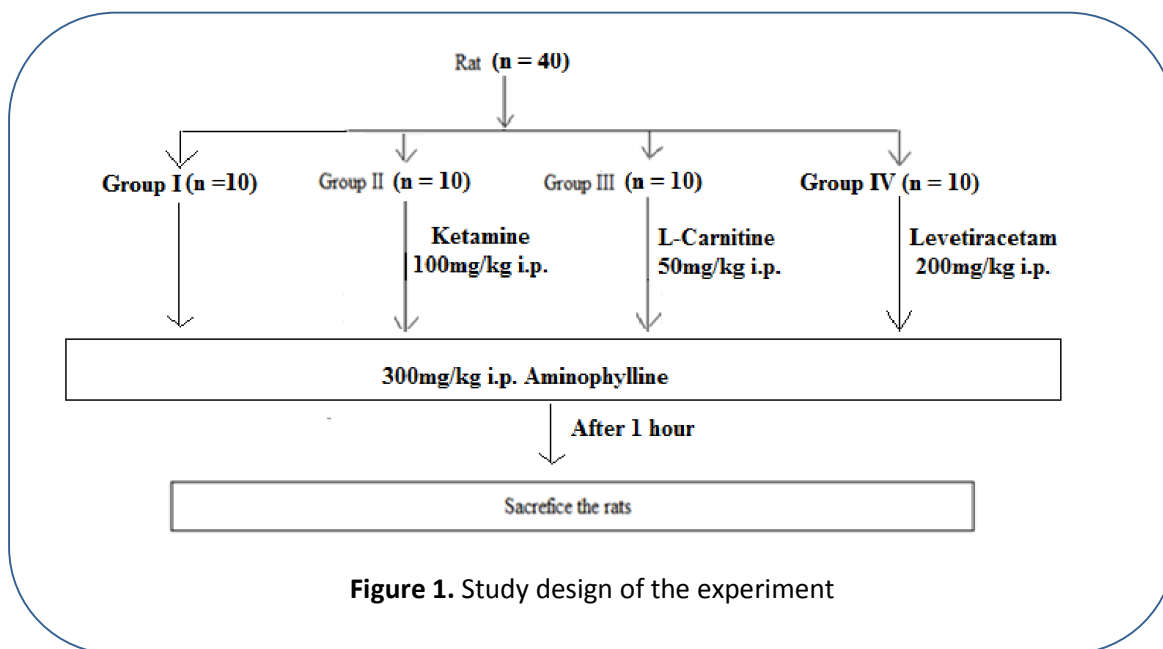
| Treatment (intraperitoneal) | Group I (n = 10) | Group II (n = 10) | Group III (n = 10) | Group IV (n = 10) |
|---------------------------------|------------------|-------------------|--------------------|-------------------|
| Seizure threshold reduction (%) | | 0 | 10* | 40* |
| Mortality (%) | | | | |
| 20 min | 80 | 80 | 60* | 10* |
| 1hour | 100 | 100 | 60* | 10* |

p values were compared to Aminophylline 300mg/kg group; p < 0.05 consider statistically significant; * signifies p < 0.001.

Table 2. Differences between the mean values of selected parameters in different area of brain of the three study groups of rats

| Parameters | Sources | Group I (Aminophylline) n = 10 | Group II (Aminophylline + Ketamine) n = 10 | Group III (Aminophylline + L-Carnitine) n = 10 | Group IV (Aminophylline + Levetiracetam) n = 10 |
|---------------------------------------|------------------|--------------------------------------|---|---|--|
| Tissue MDA (nmol/mg of protein) | Cortex | 0.95 ± 0.06 | 0.89 ± 0.08 | 0.53 ± 0.04* | 0.44 ± 0.06* |
| | Cerebellum | 0.67 ± 0.06 | 0.64 ± 0.06 | 0.35 ± 0.06* | 0.3 ± 0.08* |
| | Midbrain | 0.79 ± 0.01 | 0.76 ± 0.04 | 0.33 ± 0.05* | 0.34 ± 0.04* |
| | Basal ganglia | 1.39 ± 0.28 | 1.27 ± 0.25 | 0.47 ± 0.04* | 0.44 ± 0.07* |
| Tissue PC (mM/mg of protein) | Cortex | 0.31 ± 0.02 | 0.29 ± 0.02 | 0.18 ± 0.02* | 0.13 ± 0.02* |
| | Cerebellum | 0.26 ± 0.04 | 0.23 ± 0.03 | 0.11 ± 0.02* | 0.09 ± 0.04* |
| | Midbrain | 0.28 ± 0.02 | 0.26 ± 0.02 | 0.14 ± 0.03* | 0.18 ± 0.05* |
| | Basal ganglia | 0.56 ± 0.10 | 0.49 ± 0.11 | 0.25 ± 0.04* | 0.11 ± 0.02* |
| Cytosolic SOD (IU/mg of protein) | Cortex | 0.66 ± 0.05 | 0.71 ± 0.05 | 1.31 ± 0.04* | 0.82 ± 0.08 [#] |
| | Cerebellum | 0.59 ± 0.05 | 0.66 ± 0.11 | 0.97 ± 0.11* | 0.76 ± 0.07 [#] |
| | Midbrain | 0.44 ± 0.02 | 0.52 ± 0.23 | 0.58 ± 0.15 [#] | 0.79 ± 0.09* |
| | Basal ganglia | 0.79 ± 0.12 | 0.82 ± 0.17 | 1.29 ± 0.20* | 1.18 ± 0.07* |

Values are mean ± SD; p < 0.05 consider statistically significant; [#] signifies p < 0.05; p values were compared to Group I (Control); *signifies p < 0.001.



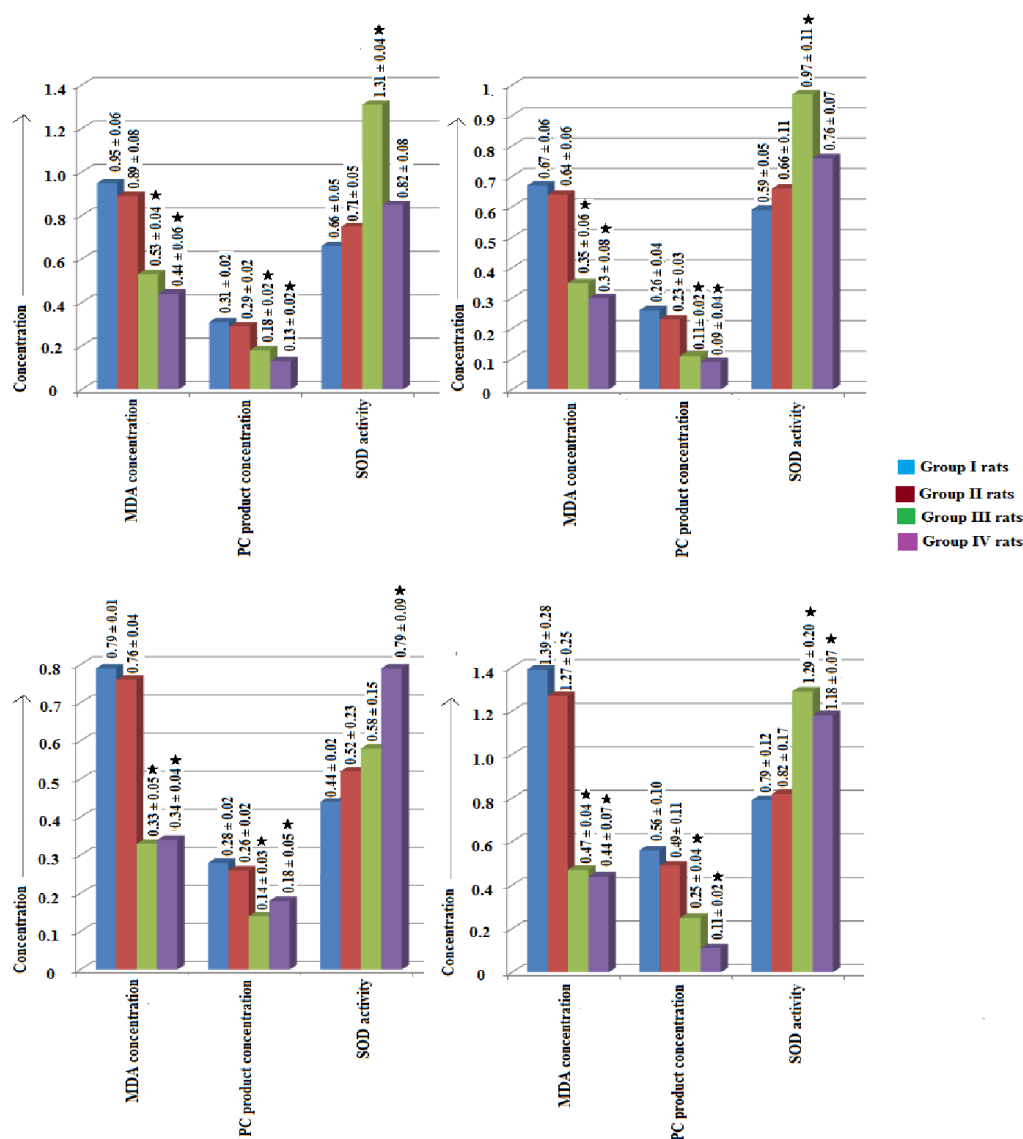


Figure 2. Histogram showing distribution of MDA, PC products and SOD in (a) Cortex, (b) Cerebellum, (c) Mid-brain, (d) Basal ganglia of Group I (Aminophylline), Group II (Aminophylline + Ketamine) Group III (Aminophylline + L-Carnitine) and Group IV (Aminophylline + Levetiracetam) rats. Asterisks indicate p<0.001; MDA, PC products and SOD in different brain area are expressed in (nmol/mg of protein), (mM/mg of protein) and (IU/mg of protein) respectively