ORIGINAL ARTICLE CODEN: AAJMBG

Antioxidant property of aminophylline in rat brain

Mrinal Pal[1*], Subinay Datta[1] and Maloy Mandol[2]

[1]Department of Biochemistry, Burdwan Medical College, Burdwan-713104 West Bengal, India and
[2]Department of Anatomy, Burdwan Medical College, Burdwan-713104 West Bengal, India

Abstract: *Background:* Free radicals being considered an important component of secondary damage during ischemia, trauma, and neurodegenerative diseases in the CNS. Aminophylline has been reported to possess free radical scavenging effect in lung tissue. But there is no information regarding aminophylline's neuroprotective effect against lipid peroxidation in the brain. *Aim:* The present study was to locate the region of brain that is particularly susceptible to oxidative damage and also aimed at investigating the antioxidant action of aminophylline in rat brain homogenates against in vitro induced lipid peroxidation. *Method:* Male Wister rats (n = 34) were randomly selected and divided into three groups. The whole rat brains all rats were homogenized in 1:10 cold Tris–HCl buffer by using homogenizer. Peroxidation was induced with ferrous iron (Fe^{2+}), ascorbate and hydrogen peroxide (H_2O_2) in Group I (n = 12). Aminophylline was added into the reaction mixtures just before the induction of peroxidation in Group III (n = 12). *Result:* The highest lipid peroxidation was obtained with Fe^{2+}, Ascorbate and H_20_2-induced group and aminophylline showed definite free radical scavenging effect on rat brain. *Conclusion:* Analysis of the results demonstrated that aminophylline has no antioxidant effect on rat brain and spinal cord homogenates in vitro. Further in vitro and in vivo studies are needed to evaluate the free radical scavenging effect of aminophylline.
Keywords: Aminophylline, Antioxidant property, Oxidative stress, lipid peroxidation.

Introduction

Oxidative stress has been considered as one of the basic events involved in cell and tissue damage. Production of free oxygen radicals being considered an important component of secondary damage during ischemia, trauma, and neurodegenerative diseases in the CNS [1-3]. The central nervous system is highly susceptible to oxidative stress. Vulnerability of brain to oxidative stress induced by oxygen free radicals seems to be due to the fact that on one hand, the brain utilizes about one fifth of total oxygen demand of body for oxidative phosphorylation to acquiring energy as compare to other cells, on other because of its relatively small antioxidant capacity [4] they cannot neutralized the toxic effect of free radicals. Further, the brain contains relatively high concentration of easily peroxidiable fatty acids [5] and it is known that certain regions of brain are highly enriched in iron, a metal that in its free form is catalytically involved in production of damaging oxygen free radical species [6-7].

So, an ROS overload damages many cellular components like cellular proteins, DNA and membrane phospholipids [8-14]. Lipid peroxidation is the consequence of ROS, the role of which is well established in the pathogenesis of a wide range of diseases like Alzheimer and Parkinson diseases [15-18] acute brain injuries such as ischemia or head trauma [19-21] and some major metabolic diseases [21].

Aminophylline, a mixture of theophylline and ethylenediamine (85:15), is a bronchodilating and antiasthmatic compound widely used in the treatment of bronchial asthma and chronic obstructive pulmonary disease [22]. Aminophylline has been reported to possess free radical scavenging effect in lung tissue [23]. But there is no information regarding aminophylline's neuroprotective effect against lipid peroxidation in the brain. So, the present study was to locate the region of brain that is particularly susceptible to oxidative damage and also aimed at investigating the antioxidant action of aminophylline in rat brain homogenates against in vitro induced lipid peroxidation. For this purpose, we used a hydroxyl radical-generating system and we

evaluated whether aminophylline was protective against the free radical-induced lipid peroxidation in different area of rat brain. As a result of lipid peroxidation, secondary end product of non enzymatic (autoxidative) fatty peroxide formation and decomposition, a great variety of aldehydes can be produced, including hexanal, malondialdehyde (MDA) and 5- hydroxynonenal [24].

Conceptually, these three facts indicate that MDA is an excellent index of lipid peroxidation. Protein carbonyls (PC) products of oxidatively modified cellular proteins are generated through a variety of mechanisms, including direct oxidation of amino acid side chains and oxidation-induced peptide cleavage. Accordingly, we carried out our present study on experimental rat models assuming that the results would have more or less similar implications in humans also.

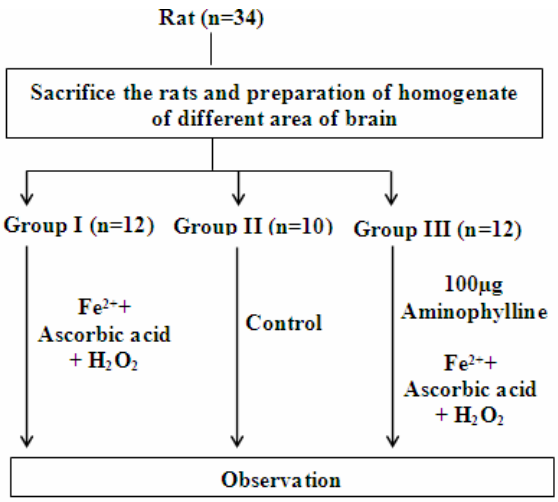
Material and Methods

Study area: The present study was an animal model based case control study undertaken in the departments of Biochemistry with the collaboration of the department of Pharmacology of Burdwan Medical College, Burdwan, West Bengal, India.

Animal: Male Wister strain albino rats (Rattus norvegicus albinus), between 1 to 2 months of age weighing 150 ± 12g, n = 34 were obtained 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 to ± 2^{o}C at a relative humidity of 45 to 50 %. All rats were acclimatized for at least 7 days before starting the experiment.

All procedures involving animals were performed in accordance with 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 started after obtaining the written consent from the concerned ethics committee [Memo No.BMC/2179/1 (5)].

Fig-1: Study Design of the experiment



Preparation of lipid substrates for peroxidation: All the rats in this study then were sacrificed by cervical dislocation. To investigate the of Aminophylline in reduction of oxidative changes in different area of brain, rat brains were dissected and segregated 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 were frozen immediately after this, at first at −20 °C and then at −70 °C, and kept under these conditions (−70 °C) until chemical analysis was performed. All assays were completed on the same day of sample collection. For homogenisation, samples were first washed and 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 X g for 10 min in a refrigerated cold centrifuge machine at 4^{0}C. Supernatants from the homogenates were collected and were estimated for MDA, PC adducts, cytosolic superoxide dismutase (Cu^{2+}-Zn^{2+}-SOD) and tissue protein immediately. The resulting 10% homogenates were used in lipid peroxidation studies [21].

Determination of lipid peroxidation: MDA were measured as an index of lipid peroxidation [25]. Peroxidation was induced with Fe^{2+} (0.02 mM), Ascorbate (1 mM) and H_20_2 (0.5 mM) in a final volume of 0.5 ml. Aminophylline at dose of 100µg was added into the reaction mixtures at indicated concentrations just before the addition of lipid substrates. MDA was measured by its reaction with thiobarbituric acid at 532 nm [26]. The brain tissue levels of MDA were calculated using a calibration curve derived from 1,1,3,3-tetraethoxypropane (Fluka, Germany) as the external calibration standard. The calibration curve was linear in range from 1.25 to 2.5 nmol/ml (r2=0.997).

Oxidation induced changes in the tissue proteins were estimated by measuring the protein carbonyl products. The method is based on the reaction of carbonyl groups with 2,4- dinitrophenylhydrazine to form a 2,4-dinitrophenylhydrazone reactive carbonyl derivate that was measured at 370 nm [27]. Estimation of cytosolic superoxide dismutase (SOD) was done by 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 NADH to nitroblue tetrazolium (NBT) by 50 % under specified conditions [28]. Tissue proteins were measured by the method of Lowry et al [29] that involved reaction of proteins in tissue homogenates with alkaline copper sulphate followed by another reaction with Folin's phenol reagent (SRL, India) against a standard curve prepared form bovine serum albumin (Merck, Germany). All photometric measurements were performed in Dual beam spectrophotometer (UV 5704SS).

Statistical analysis: Data obtained were analysed for significance of differences of means between the control with Group II and Group III rats by independent t test. For all tests 'p' value was considered to be significant if it was less than 0.05 at a confidence level of 95 %. All statistical analyses were performed with the help of the statistical software package SPSS version 11.5 for Windows.

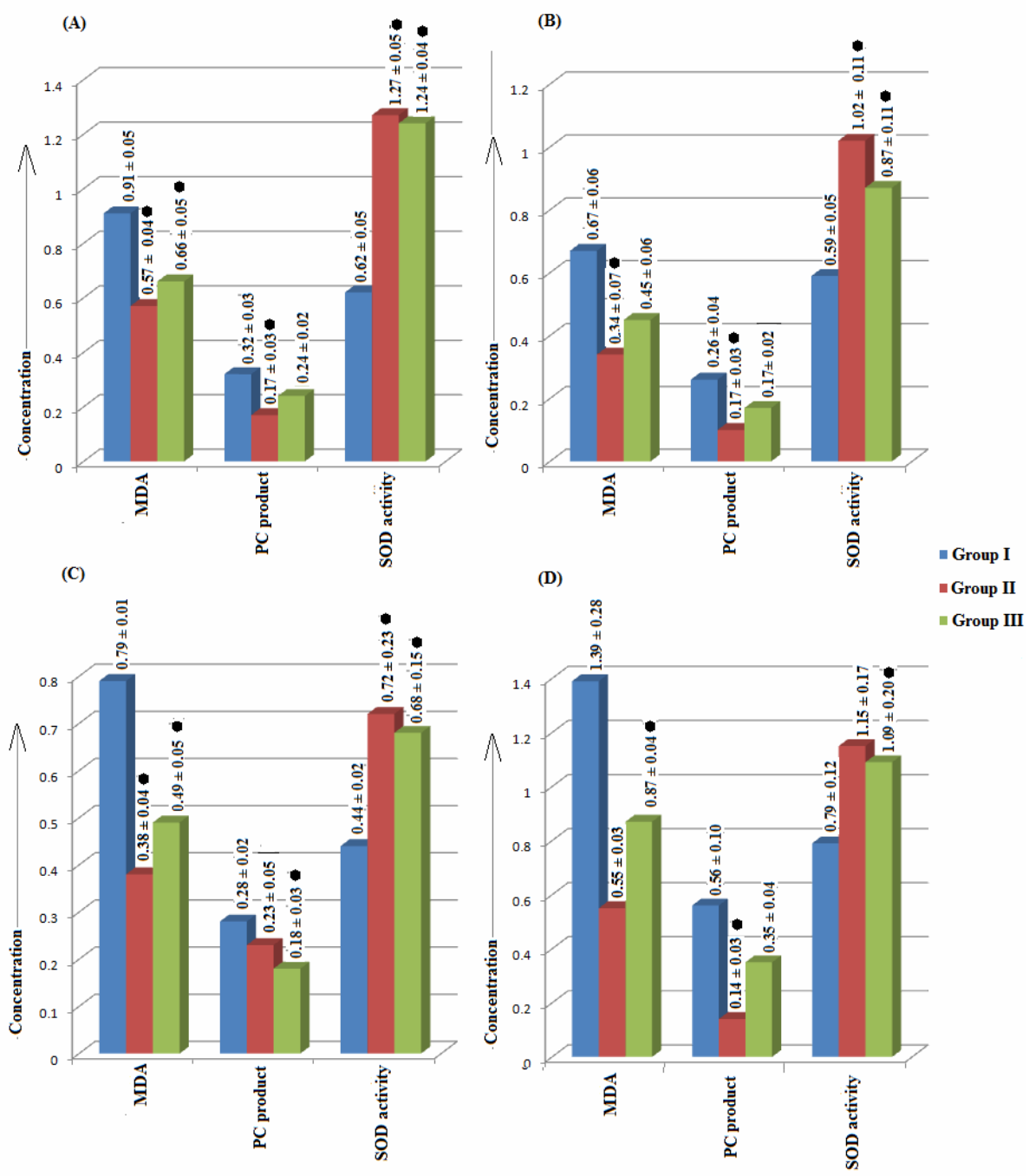
Results

Significance of differences between the means of oxidative parameters in different area of brain between the Group I, II and III – Independent 't' test. In Table 1 and Figure 2, it is shown that MDA and PC product concentration were significantly higher in all area areas of the brain in the peroxidation group than control group. At the same time the intracellular antioxidant enzyme, Cu^{2+}-Zn^{2+}-SOD was found to be significantly compromised in peroxidation group than control. To compare the efficiency in reduction of oxidative stress by Aminophylline, Independent sample t test was performed between Group I rats and Group III (Table 1), it was observed that MDA and PC product formation were significantly suppressed and SOD activity was improved in different areas of brain by Aminophylline administration ($P < 0.001$, Figure-2) in Group III rats.

Table-1: Differences between the mean values of selected parameters in different area of brain of the three study groups of rats

Parameters	Sources	Group I (Peroxidation) n = 12	Group II (Control) n = 10	Group III (Aminophylline) n = 12	Group II vs Group III	Group I vs Group II	Group I vs Group III
Tissue MDA (nmol/mg of protein)	Cortex	0.91 ± 0.05	0.57 ± 0.04	0.66 ± 0.05	p> 0.05	p<0.001	p<0.001
	Cerebellum	0.67 ± 0.06	0.34 ± 0.07	0.45 ± 0.06	p> 0.05	p<0.001	p=0.009
	Midbrain	0.79 ± 0.01	0.38 ± 0.04	0.49 ± 0.05	p> 0.05	p<0.001	p<0.001
	Basal ganglia	1.39 ± 0.28	0.55 ± 0.03	0.87 ± 0.04	p> 0.05	p<0.001	p<0.001
Tissue PC (mM/mg of protein)	Cortex	0.32 ± 0.03	0.17 ± 0.03	0.24 ± 0.02	p> 0.05	p<0.001	p=0.033
	Cerebellum	0.26 ± 0.04	0.10 ± 0.03	0.17± 0.02	p> 0.05	p<0.001	p=0.028
	Midbrain	0.28 ± 0.02	0.23 ± 0.05	0.18 ± 0.03	p> 0.05	p=0.043	p<0.001
	Basal ganglia	0.56 ± 0.10	0.14 ± 0.03	0.35 ± 0.04	p> 0.05	p<0.001	p=0.015
Cytosolic SOD (IU/mg of protein)	Cortex	0.62 ± 0.05	1.27 ± 0.05	1.24 ± 0.04	p> 0.05	p<0.001	p<0.001
	Cerebellum	0.59 ± 0.05	1.02 ± 0.11	0.87 ± 0.11	p = 0.036	p<0.001	p<0.001
	Midbrain	0.44 ± 0.02	0.72 ± 0.23	0.68 ± 0.15	p> 0.05	p<0.001	P<0.001
	Basal ganglia	0.79 ± 0.12	1.15 ± 0.17	1.09 ± 0.20	p>0.05	p<0.001	P<0.001
Values are mean ± SD; p < 0.05 consider statistically significant							

Fig-2: Histogram showing distribution of MDA, PC products and SOD in (a) Cortex, (b) Cerebellum, (c) Mid-brain, (d) Basal ganglia of Group I (Peroxidation), Group II (Control) and Group III (Aminophylline) rats.



Discussion

Free oxygen radicals are reactive species which can destroy the tissues via lipid peroxidation of the cell membranes [30-31]. Excessive generation of free oxygen radicals causes DNA damage, lipid peroxidation and inactivation of proteins and finally leads to severe tissue injury [32-35]. Pharmacological agents who can prevent or reduce free oxygen radical mediated toxicity can be clinically useful. The present study by using the Fe^{2+}-Ascorbic acid-H_20_2 system oxidant conditions was generated. This system generates the most reactive radical species, the hydroxyl radical, through the reaction of ferrous iron with H_20_2 (Fenton reaction).

The iron is oxidized to its ferric form and in the presence of ascorbate as a reducing agent, ferrous iron is recovered from its oxidized form. As a consequence, hydroxyl radical generation and peroxidation persists. Then on administration of Aminophylline it was revealed that aminophylline efficiently inhibit lipid peroxidation in rat brain that was indicated by significantly suppression of MDA and PC product as well as improvement of SOD activity in different areas of brain in Group III rats. Aminophylline is a salt composed of two molecules of theophylline and one molecule of ethylenediamine. Therapeutic concentrations of aminophylline, are capable of antagonizing hypochlorous acid (HOCl) and moreover Aminophylline at lower concentration was found to be effectively scavenging OH radical, because of its ethylenediamine component [36]. Theophylline has antioxidant effects also due to its non-selective phosphodiesterase inhibitory properties in human neutrophils [37-38].

Conclusion

Analysis of the results demonstrated thataminophylline has no antioxidant effect on rat brain homogenates in vitro. Further in vitro and in vivo studies are needed to evaluate the free radical scavenging effect of aminophylline.

Acknowledgement

Authors are thankful to Dr. Keya Pal and Dr. Supreeti Biswas of Burdwan Medical College and Hospital for constant support & inspiration.

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*All correspondences to: Dr. Mrinal Pal, Assistant Professor, Department of Biochemistry, Burdwan Medical College, Burdwan-713104, West Bengal, India. E-mail: mrinalpal77@rediffmail.com