The Antioxidant Effect of Aminophylline in Rat Brain and Spinal Cord Homogenates

Rat Beyin ve Omurilik Homojenatlarında Aminofilinin Antioksidan Etkisi

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Abstract: ***Object:*** Free oxygen radicals play a major role in the pathophysiology of central nervous system (CNS) trauma, neurodegeneration and aging. Aminophylline, an antiasthmatic drug, was shown to exert some free radical scavenging effect on lung tissue. The purpose of this study was to investigate the free radical scavenging effect of aminophylline on CNS tissues. The antioxidant action of different doses of aminophylline was studied under oxidant conditions in vitro.

Methods: The whole rat brains and spinal cords obtained from male Wistar rats were homogenized in 1:10 cold potassium phosphate buffer by using a dounce homogenizer. Peroxidation was induced with ferrous iron (Fe^{++}), ascorbate (Asc) and hydrogen peroxyde (H_2O_2). Aminophylline was added into the reaction mixtures just before the addition of lipid substrates. Thiobarbituric acid-reacting substances (TBARS) were measured as an index of lipid peroxidation.

Results: The highest lipid peroxidation was obtained with Fe^{++}, Asc and H_2O_2-induced group. Addition of aminophylline showed no free radical scavenging effect on rat brain and spinal cords.

Conclusion: Analysis of the results demonstrated that

Özet: ***Amaç:*** Serbest oksijen radikalleri merkez sinir sistemi travması, nörodejenerasyon ve yaşlanmanın patofizyolojisinde önemli rol oynamaktadır. Bir antiastmatik olan aminofilin akciğer dokusunda serbest radikal tutucu etki göstermektedir. Bu çalışmanın amacı aminofilinin merkez sinir sisteminde serbest radikal tutucu etkisini incelemektir. Aminofilinin farklı dozları in vitro antioksidan koşullarda çalışıldı.

Metod: Dişi Wistar ratlardan elde edilen tam beyin ve omurilik dokuları 1:10 soğuk fosfat tamponunda homojenizatör ile homojenize edildi. Peroksidasyon ferröz demir (Fe^{++}), askorbat (Asc) ve hidrojen peroksit (H_2O_2) ile indüklendi. Aminofilin reaksiyon karışımına lipid substratların eklenmesinden önce ilave edildi. Tiyobarbitürik asit reaktif maddeler (TBARS) lipid peroksidasyonunun bir indeksi olarak ölçüldü.

Bulgular: En yüksek lipid peroksidasyonu Fe^{++}, Asc ve H_2O_2 indükleme grubunda elde edildi. Karışıma aminofilin ilave edilmesi rat beyin ve omurilik dokularında peroksidasyona karşı koruyucu etki göstermedi.

Sonuç: Sonuçların analizi aminofilinin rat beyin ve omurilik homojenatlarında antioksidan etkisinin

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.

Key words: Aminophylline, antioxidant, brain, lipid peroxidation, spinal cord

olmadığını gösterdi. Aminofilinin antioksidan özelliğini değerlendirmek için in vitro ve in vivo ileri çalışmalara gereksinim vardır.

Anahtar Kelimeler: Aminofilin, antioksidan, beyin, lipid peroksidasyon, omurilik

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 (1,7,11). Re-oxygenation of the ischemic tissues results in generation of free oxygen radicals. The generation of free oxygen radicals results in lipid peroxidation of cell membrane, damage of DNA and other subcellular organels and subsequently cell death (8,12,22). The antioxidant activity of drugs have great clinical interest because of the importance of preventing or reducing the free radical-mediated toxicity in CNS.

There are many clinical and experimental investigations about the free radical scavenging properties of various antiasthmatic drugs in the literature such as salbutamol, terbutaline, fenoterol and theophylline (10,19,24). Aminophylline, a mixture of theophylline and ethylenediamine (85:15%), is a bronchodilatating and antiasthmatic compound widely used in patients with acute respiratory insufficiency (3,17,23). Aminophylline has been reported to possess free radical scavenging effect in lung tissue (18). Currently there is no information regarding aminophylline's neuroprotective effect against lipid peroxidation in the rodent brain and spinal cord. In the present study, we aimed at investigating the antioxidant action of aminophylline in rat brain and spinal cord 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.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid (TBA), ascorbic acid (Asc) and hydrogen peroxide (H2O2) were from Sigma Chemical Co. (St Louis, MO, USA). Trichloroacetic acid (TCA) and ferrous sulfate were from Merck (Germany). Aminophylline was from Turfarma (Turkey).

Preperation of lipid substrates for peroxidation

Brain and spinal cord homogenates were used to investigate the antioxidant actions of aminophylline. Two male, 2 month old Wistar rats, approximately 200 g were used for tissue preperation. The rats were anesthetized with combination of 60 mg/kg ketamine hydrochloride (Parke Davis, Istanbul,) and 10 mg/kg xylazine (Bayer Birleşik Alman İlaç Fabrikaları A.Ş., Istanbul), and placed prone to the operation table. Brains were obtained following wide craniectomy and spinal cords were obtained following C_3-T_{12} laminectomy. Dura was removed from the neural tissues with scalpel, and the samples were rinsed with physiologic saline. The whole rat brains and spinal cords were homogenized in 1:10 (w:v) cold potassium phosphate buffer (25 mM, pH 7.4) by using a dounce homogenizer. The resulting 10% homogenates were used in lipid peroxidation studies (6).

Determination of lipid peroxidation

Thiobarbituric acid-reacting substances (TBARS) were measured as an index of lipid peroxidation (2). Peroxidation was induced with Fe^{++} (0.02 mM), Asc (1 mM) and H_2O_2 (0.5 mM) in a final volume of 0.5 ml. Aminophylline was added into the reaction mixtures at indicated concentrations just before the addition of lipid substrates. The reaction mixture was incubated for 30 min at room temperature. At the end of incubation, 0.5 ml of TCA (10%) was added to stop the reaction. Samples were centrifuged at 1500 x g for 10 min and 0.5 ml of supernatant was mixed

with 0.5 ml of TBA (0.67%). Tubes were placed into boiling water for 15 min. After cooling the tubes, the absorbance of the samples was measured at 532 nm.

RESULTS

Fe^{++}, Asc and H_2O_2 were used to induce lipid peroxidation. The ferrous iron was the most effective agent in induction, although each component, when used alone, was able to induce lipid peroxidation. Combination of two agents showed that the highest peroxidation was obtained with Fe^{++} and Asc combination. The overall highest peroxidation levels were measured in the presence of Fe^{++}, Asc and H_2O_2 (Table 1). The iron-Asc-H_2O_2 system was very effective for generating oxidant conditions.

The results of spectrophotometry showed that there was a gradual increase in lipid peroxidation levels with increasing amounts of aminophylline. The absorbance of the samples is shown in Table 2. Results are expressed as the mean ± SD.

DISCUSSION

In intense ischemic state followed by re-oxygenation could result in irreversible organ damage (21). Mitochondrial damage due to calcium overload of the cells and excessive generation of free oxygen radicals are the underlying mechanisms of reperfusion injury (4). Increased cytoplasmic calcium results in the production of reactive oxygen species (15). Free oxygen radicals are reactive species which can destroy the tissues via lipid peroxidation of the cell membranes (13,14). Excessive generation of free oxygen radicals causes DNA damage, lipid peroxidation and inactivation of proteins and finally leads to severe tissue injury (8,9,12,22). Pharmacological agents which can prevent or reduce free oxygen radical mediated toxicity can be clinically useful.

Although β_2-agonists seem to have a direct oxidant scavenger function, which antiasthmatic drugs have antioxidant effect remains speculative. Glissen et al. (10) demonstrated that β_2-agonists have a potent antioxidative function in H_2O_2-mediated cytotoxicity in vitro. They also suggested that corticosteroids and theophylline had no antioxidant effect. Aminophylline is a salt composed of two molecules of theophylline and one molecule of ethylenediamine and is a well known antiasthmatic drug (3,17,23). Kang et al. reported that aminophylline exert some antioxidant activity in vitro (16). Similarly, Lapenna et al. reported the free radical scavenging effect of aminophylline, but not theophylline in lung epithelial tissue (18). They showed that both aminophylline and theophylline are scavengers of hydroxyl radical (OH), but they are ineffective against superoxide anion and hydrogen peroxide. Aminophylline was found to be effective in lower concentrations, because of ethylenediamine's marked OH scavenging activity. Moreover, they demonstrated that therapeutic concentrations of aminophylline, but not of theophylline, are capable of antagonizing hypochlorous acid (HOCl); this effect was entirely due to presence of ethylenediamine. They hypothesized that antioxidant properties of aminophylline, partly or totally, is attributable to ethylenediamine which is present in the aminophylline molecule.

Mahomed et al. suggested that theophylline has antioxidant effects due to its phosphodiesterase inhibitory properties in human neutrophils (19). Aminophylline, also a non-selective phosphodiesterase inhibitor, inhibits phosphodiesterase enzyme that metabolize and inactivate cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) (20). Increase in the intracellular concentration of either cAMP or cGMP results in relaxation of airway smooth muscle. It was also expected that inhibition of these enzymes results in reduction of free radicals in the tissues. In the present study, aminophylline itself does not have antioxidant effect in vitro. But its effect on phosphodiesterase enzyme may results in decrease in tissue free radical levels after traumatic or ischeamic insult in vivo. However, translocation of intracellular calcium and the antagonistic blockade of adenosine receptors are the other pharmacological actions of aminophylline (5).

The aim of our present study was to investigate whether aminophylline had an antioxidant action. We analyzed the effect of aminophylline toward lipid peroxidation in vitro,

using the Fe^{++}-Asc-H_2O_2 system to generate oxidant conditions. This system generates the most reactive radical species, the hydroxyl radical, through the reaction of ferrous iron with H_2O_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. In this study, when Fe^{++}-Asc-H_2O_2 was added, levels of lipid peroxidation products in the homogenates were significantly increased (Table I). The Fe^{++}-Asc-H_2O_2 system was found to be very effective for generating oxidant conditions (12-14). Since a hydroxyl radical scavenging effect was previously described for aminophylline (18), different concentrations of the drug were added to the homogenates in order to evaluate a possible protective action. The results showed that aminophylline did not inhibit lipid peroxidation in rat brain and spinal cord homogenates (Table II). We concluded that aminophylline was not an efficient scavenger against Fe^{++}-Asc-H_2O_2 -induced radical generation, at least in our experimental conditions.

Table I: Table shows the basal TBARS levels of the rat brain and spinal cord homogenates. Increase in tissue TBARS levels are seen with induction of one, two or all of Fe^{++}, Asc and H_2O_2. The best induction was observed with addition of all chemicals. The values are the absorbances of the samples was measured at 532 nm., and are exspressed as the mean ± SD.

	Brain	Spinal cord
Basal	40,48±0,96	17,01±0,00
Fe^{++}	288,44±7,43	158,79±0,28
Asc	58,28±6,98	31,48±0,28
H_2O_2	174,24±1,92	107,36±4,57
Fe^{+} Asc	434,92±1,54	307,61±8,05
Fe^{+} H_2O_2	343±13,17	142,95±0,73
Asc$^{+}H_2O_2$	283,36±0,48	303,70±14,13
Fe^{+}Asc^{+} H_2O_2	715,93±36,17	629,30±6,47

In summary, the role of free oxygen radicals in severe tissue injury, especially in ischemia-reperfusion induced organ damage and trauma to CNS, is well known. Importance of preventing and reducing the free oxygen radical induced secondary neuronal damage must be considered in various clinical conditions. Pharmacological agents which have radical scavenging properties are needed to be studied. Further in vitro and in vivo studies are needed to evaluate the free radical scavenging effect of aminophylline.

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REFERENCES

1. Anderson DK, Hall ED: Pathophysiology of spinal cord trauma. Ann Emerg Med 22:987-992, 1993
2. Ames BN, Catchard R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. Proc Natl Acad Sci USA 78:6858-6862, 1978

Table II: Table shows the effect of aminophylline on free radicals in brain and spinal cord homogenates. The values are the absorbances of the samples was measured at 532 nm, and are exspressed as the mean ± SD.

	Basal	Induction	Aminophylline 25 μM	Aminophylline 50 μM	Aminophylline 100 μM
Brain	40,48±0,96	715,93±36,17	762,67±42,58	898,77±85,78	1089,05±169,25
Spinal cord	17,01±0,00	629,30±6,47	781,64±33,78	888,00±40,67	957,05±30,51

3. Bernaskova K, Mares P. Proconvulsant effect of aminophylline on cortical epileptic after discharges varies during ontogeny. Epilepsy Res 39:183-190, 2000
4. Cheung JY, Bonventre JV, Malis CD, Leaf A. Calcium and ischemic injury. N Engl J Med 26:1670-1676, 1986.
5. Dani C, Bertini G, Reali MF, Tronchin M, Wiechmann L, Martelli E, Rubaltelli FF. Brain hemodynamic changes in preterm infants after maintenance dose caffeine and aminophylline treatment. Biol Neonate 78:27-32, 2000
6. Demirpence E, Caner H, Bavbek M, Kılınc K. Antioxidant action of the antiarrhythmic drug mexiletine in brain membranes. Jpn J Pharmacol 81:7-11, 1999
7. Dumont RJ, Okonkwo DO, Verma S, Hurlbert J, Boulos PT, Ellegala DB, Dumont AS: Acute spinal cord injury, Part I: pathophysiologic mechanisms. Clin Neuropharmacol 24:254-264, 2001
8. Freeman BA, Crapo JD: Free radicals and tissue injury. Lab Invest 47:412-426, 1982
9. Gillissen A, Jaworska M, Scharling B, van Zwoll D, Schultze-Werninghaus G. Beta-2-agonists have antioxidant function in vitro. 1. Inhibition of superoxide anion, hydrogen peroxide, hypochlorous acid and hydroxyl radical. Respiration 64(1): 16-22, 1997
10. Gillissen A, Wickenburg D, van Zwoll D, Schultze-Werninghaus G. Beta-2-agonists have antioxidant function in vitro. 2.The effect of B2 agonists on oxidant-mediated cytotoxycity and on superoxyde anion generated by human polymorphonuclear leucocytes. Respiration 64(1): 23-28, 1997
11. Hall ED. Lipid antioxidant in acute central nervous system injury. Ann Emerg Med 22:1022-1027, 1993
12. Halliwell B, Aruoma OI: DNA damage by oxygen-derived species. FEBS Letter 281:9-19, 1991
13. Halliwell B, Chiroco S. Lipid peroxidation: Its mechanism, measurement and significance. Am J Clin Nutr 57(Suppl):15-25, 1993
14. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol 186:1-83, 1990
15. Hearse DJ, Tosaki A. Free radicals and calcium: Simultaneous interacting triggers as determinants of vulnerability to reperfusion-induced arrhythmias in the rat heart. J Mol Cell Cardiol 20:213-223, 1988
16. Kang MY, Tsuchiya M, Packer L, Manabe M. In vitro study on antioxidant potetial of various drugs used in the perioperative period. Acta Anaesthesiol Scand 42(1):4-12, 1998
17. Kawai H, Kokubun S, Matsumoto T, Kojima J, Onodera K. Pharmacokinetic study of theophylline in dogs after intravenous administration with and without ethylenediamine. Methods Find Exp Clin Pharmacol 22(3):179-184, 2000
18. Lapenna D, De Gioia S, Mezzetti A, Ciofani G, Festi D, Cuccurullo F. Aminophylline: could it act as an antioxidant in vivo?. Eur Clin İnvest 25:464-470, 1995
19. Mahomed AG, Theron AJ, Anderson R, Feldman C. Anti-oxidative effects of theophylline on human neutrophils involve cyclic nucleotides and protein kinase A. Inflammation 22(6):545-557, 1998
20. Matsuda F, Sugahara K, Sugita M, Sadohara T, Kiyota T, Terasaki H. Comparative effect of amrinone, aminophylline and diltiazem on rat airway smooth muscle. Acta Anaesthesiol Scand 44:763-766, 2000
21. Severina MJ, Chintala MS. Eficacy and mechanisms of dopexamine in the prevention of ischemia-reperfusion induced organ damage:role of oxygen free radicals. Clin and Exp Hypertens 19:181-190, 1997
22. Slater FT: Free-radical mechanisms in tissue injury. Bichem J 222:1-15, 1984
23. Yamana K, Sano T, Suzuki N, Kojima J, Onodera K. Effects of intravenous theophylline, aminophylline and ethylenediamine on antigen-induced bronchoconstriction in Guinea pigs. Methods Find Exp Clin Pharmacol 22(2):97-100, 2000
24. Zwicker K, Damerau W, Dikalow S, Scholtyssek H, Schimke I, Zimmer G. Superoxyde radical scavenging by phenolic broncodilatators under aprotic and aqueous conditions. Biochem Pharmacol 56:301-305, 1998