# THE PROTECTIVE EFFECTS OF VITAMIN E ON ISCHEMIC NEURONAL DAMAGE IN RATS

Kanokwan Tilokskulchai*, Jantana Shuprisha**, Rachawan Limviwatkul**, Suparp Nualplub** and Ratana Ninturk*

**Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, THAILAND* ***Department of Physiology, Faculty of Science, Prince of Songkla University, Songkla, THAILAND*

## ABSTRACT

Antioxidant vitamins (i.e. vitamin E and vitamin C) in large dosages are increasingly common self-prescribed drugs. This increase in vitamin use has, perhaps, been promoted by the claim that vitamin may provide protection from ischemic brain damage or prevent age-associated diseases induced by free radicals and lipid peroxidation. Apart from such legitimate uses of mega-dosages of vitamins, at present there is no conclusive evidence to support the recommendation of such use. Therefore, the purpose of the present study is to investigate the efficacy of short-term and long-term vitamin E supplements in protecting brain damage from transient global cerebral ischemia in Spraque-Dawley rats. The present study was designed to examine vitamin E's effect on histological outcome 7 days after transient global cerebral ischemia in the treated and untreated animals. Global cerebral ischemia was induced by 30 minutes bilateral occlusion of common carotid arteries with lowering mean arterial blood pressure to 60-80 mmHg before occlusion. For the short-term study, animals were divided into two groups. Group 1 was subjected to cerebral ischemia with no medication and group 2 was fed daily with vitamin E (30 mg/kg) from 2 weeks before to one week after ischemic insult. For the long-term study, global cerebral ischemia was induced in group 1 at the age of 15-19 months with no medication. Group 2 was fed daily with vitamin E (30 mg/kg) for 12-15 months before ischemic insult beginning from the age of 3 months. The result demonstrated that the mean percentage of cell death in the 2 weeks supplement group ($17.8\pm0.8$) was significantly less than that of the untreated group ($28.0\pm0.8$)($P<0.05$). However, the cell death in the long-term(12-15 months) vitamin E supplement group was not significantly different from the untreated group. The present study, therefore, revealed that short-term vitamin E supplement provided partial protective effect from acute ischemic brain damage. In contrast, our results also showed that long-term vitamin E supplement could not influence the outcome damage induced by transient ischemic attack. Therefore, vitamin E supplement should not be recommended in healthy elderly population. It should be reserved for individuals with documented deficiency or who are at risk only.

**Key words** : antioxidant, vitamin E, ischemic brain damage

Address correspondence and reprint requests to: Kanokwan Tilokskulchai, PhD. Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, THAILAND. Tel/Fax: 4197579

## INTRODUCTION

Free radicals and lipid peroxidation have been suggested as important causative agents of several human diseases including ischemic cell death and age-related degenerative disease[1-4]. Free radicals are normally produced during mitochondrial respiration, autooxidation of a variety of biological molecules and chemicals. Since free radicals are highly reactive, a series of cellular defense mechanisms have been evolved to prevent uncontrolled radical reactions. However, under pathologic condition or oxidative stress such as ischemia/ reperfusion, larger amount of oxygen free radicals are formed than normal which can overwhelm the defense of the cell. The excess free radicals may induce lipid peroxidation, causing neuronal damage resulting in delayed neuronal death at selective vulnerable areas of the brain[5]. Furthermore, brain tissue is particularly susceptible to free radical attack due to its high metabolic rate and high concentration of polyunsaturated fatty acids which are highly susceptible to oxidation. Therefore, toxic free radicals may be one of the major potential causes of age-related destruction of neuronal tissue[6]. Evidences suggest that vitamin E which is well accepted as the most effective natural lipid-soluble antioxidant or free radical scavenger, could theoretically be helpful where oxidization and free radical formation are the initiating events in disease and aging processes[7-8]. Therefore, elderly subjects who are more susceptible to oxidative stress, may gain benefit from the antioxidant protection provided by vitamin E. Vitamin E supplement in large dose is increasingly common in elderly population in an attempt to halt the normal aging process or prevent and cure diseases. Despite it has been realized that antioxidant will not delay aging in healthy elderly people, vitamin E supplement may be given to prevent some diseases such as acute ischemic attack[8].

The present study, therefore, is based on an attempt to investigate two problems. First is to assess whether short-term supplement of vitamin E in young adult rats can provide protection against brain damage from abrupt increase in free radicals by acute cerebral ischemia. Second is to observe whether long-term supplement of vitamin E in rats from the age of 3 months until 15-19 months can protect brain from damage induced by acute ischemic attack or not.

## MATERIALS AND METHODS

*Experimental model*

Forty Spraque-Dawley rats of both sexes were used in the present study. The animals were divided into two groups to study the effects of short-term and long-term vitamin E supplements on protection the brain damage from transient global cerebral ischemia. Global cerebral ischemia was induced by bilateral occlusion of common carotid arteries for a period of 30 minutes in anesthetized animals (Nembutal 40-45 mg/kg). Anaesthesia was maintained throughout the experiment by intravenous injection of supplementary dose of nembutal (usually about 4-5 mg/kg/hr). Occlusion of the arteries was obtained by gently tightening polyethylene (PE) loops around the common carotid arteries. Collateral blood supply to the brain was prevented by lowering systolic blood pressure to less than 100 mmHg before induction of ischemia. After 30 minutes of arterial occlusion, the polyethylene loops were loosened to allow recirculation of blood to the brain. Animals were then allowed to recover from anesthesia and returned to their cages.

*Experimental procedure*

Short-term supplement of vitamin E

Thirty male rats, weighing from 200-250 gm, were used. Animals were randomly divided into two groups. Group 1 included 15 rats for control. Global cerebral ischemia was induced in this group of animals without vitamin E supplement. Group 2, included 18 rats for experiment, was fed with vitamin E daily (30 mg/kg) from 2 weeks before to one week after ischemic insult.

Long-term supplement of vitamin E

Ten female rats were raised for 12-16 months, from the age of 3 months. Animals were randomly divided into two groups. Group 1 included 5 rats control. Global cerebral ischemia was induced in this group at the age of 15-19 months without vitamin E supplement. Group 2, treated group, included 5 rats, was fed daily with vitamin E (30 mg/kg) for 12-16 months before ischemic insult beginning from the age of 3 months.

*Histological evaluation*

One week after ischemic insult, rats were reanesthetized and sacrified. The rat's brain was prewashed with 50 ml saline and fixed by transcardial perfusion with 250 ml of 10% formalin in 0.1 M phosphate buffer (pH

7.4). The brain was removed and postfixed in the same perfusate at 4°C for one week. Following fixation, the brain was cut coronally into 6 pieces of 2-3 mm thickness and embedded in paraffin by using automatic tissue processor. Coronal sections of the forebrain were cut into 6 μm thicknesses and stained with hematoxylin and eosin (H and E). The paraffin sections were examined under light microscope. All microscopic sections were analyzed by one observer who was blinded to the experimental conditions. Estima-tion of the extent of neuronal damage in each brain was assessed by counting total number of both normal cells and ischemic damaged cells in the specified regions of the frontal and parietal areas. Four randomized microscopic fields in the frontal region (two fields from left side and two fields from right side) and eight randomized fields in the parietal region (four fields from left side and four fields from right side) were chosen (Figure 1). Total number of normal cells and ischemic damaged cells in each fields were noted. The extent of ischemic damage in both areas of each animal was expressed as percentage of ischemic cell death, i.e. Percentage of dead cells in the fronto-parietal area of one animal = (Sum of total number of dead cells from 12 fields in the fronto-parietal region / Sum of total number of both normal and dead cells in the same region) x 100

*Analysis of results*

All data are reported as mean and standard error of the mean. Differences between groups were analysed by using unpaired t-test. P value of $<0.05$ was considered as statistically significant difference.

## RESULTS

Physiological conditions of all animals which survived after the surgery were in good health. No obvious motor deficit or any reduction in degree of alertness was observed in each group of animals studied. Global cerebral ischemia was induced by bilateral common carotid occlusion with lowering mean arterial blood pressure to 70-74 mmHg. Microscopic examination of neuronal damage revealed that cell death was found mostly in the frontal and parietal cortex with lesser extents observed in the regions of hippocampus, striatum, thalamus and occipital cortex. Hence, the frontal-parietal area was chosen to estimate the degree of neuronal damage in each group.

*Effects of short-term supplement of vitamin E*

Estimation of the amount of ischemic damage expressed as percentage of number of dead cells in the fronto-parietal area of each animal was summarized in Table 1. In the treated animals, vitamin E was administered orally (30 mg/kg) everyday from two weeks before to one week after ischemic episode. The result demonstrated that mean percentage of cell death in vitamin E treated group ($17.8 \pm 0.8$) was significantly less than the untreated group ($28.0 \pm 0.8$) ($P<0.05$). The present result revealed that vitamin E supplement could provide partial protection against brain damage from oxidative stress induced by global cerebral ischemia.

*Effect of long-term supplement of vitamin E*

The percentages of cell death in each animal of the untreated group and the treated group were shown in Table 2. Long-term vitamin E supplement slightly attenuated the brain damage resulting from transient cerebral ischemia. However, the reduction in brain damage did not reach statistically significant level. It is interesting to note that the mean cell death of the young untreated group ($28.57 \pm 1.3$) was significantly lower than that of the old-untreated group ($33.67 \pm 3.02$). The present result,therefore, revealed that long-term supplement of vitamin E failed to protect the brain from acute ischemic damage.

## DISCUSSION

The present results demonstrated partial protective effect of short-term vitamin E supplement against neuronal damage resulting from transient forebrain ischemia in young rats. In contrast, our study showed that long-term supplement of vitamin E failed to protect the brain against damage from transient cerebral ischemia.

The present evidence of partial protective effect of short-term vitamin E supplement in young animals is agreed with several other studies. Fujimoto demonstrated that vitamin E given intravenously helped in the recovery of brain electrical activity 3 hours after recirculation[9]. In addition, Yamamoto showed that intravenous injection of alpha-tocopherol prior to ligation of carotid arteries significantly suppressed the rises in lipid peroxides both in the brain and serum,

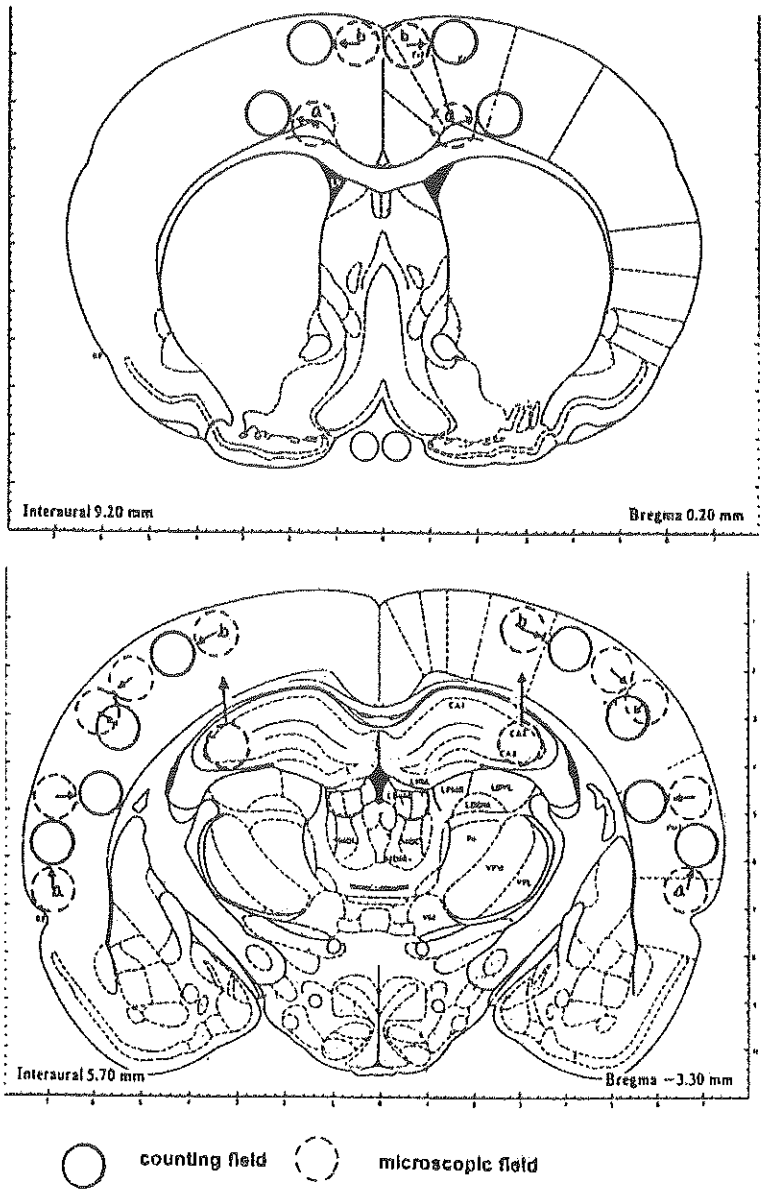


**Figure 1** Semidiagramatic drawing of coronal section demonstrating the location of counting fields used in the frontal region (upper) and parietal region (lower). In order to place the counting field in a systematic randomized manner and confined only in the frontal and parietal areas, the microscopic fields were always started from fields a and b, and then moved further to the boundary between areas (as indicated by arrows). The counting fields chosen (as indicated by solid circle) were started from the one next to 'a' and 'b' and then omitted the next one and chose the further one, respectively.

**Table 1** Percentage of cell death in the fronto-parietal area of the short-term vitamin E supplement group. All data are expressed as percentage of ischemic damage cells of individual animal, mean percentage and standard error of the mean of each group. Differences between groups are identified by using unpaired t-test and the level of significance of the difference is * P< 0.05.

| No. | Group1 | Group 2 |
|---|---|---|
| 1 | 23.3 | 12.7 |
| 2 | 23.8 | 14.0 |
| 3 | 25.6 | 14.3 |
| 4 | 25.6 | 14.9 |
| 5 | 25.7 | 15.7 |
| 6 | 25.8 | 16.2 |
| 7 | 27.9 | 17.7 |
| 8 | 28.0 | 17.8 |
| 9 | 28.5 | 18.3 |
| 10 | 28.5 | 18.9 |
| 11 | 28.9 | 19.9 |
| 12 | 30.4 | 20.9 |
| 13 | 32.3 | 21.1 |
| 14 | 32.8 | 21.4 |
| 15 | 34.0 | 24.0 |
| Mean | 28.0 | 17.8* |
| SD | 3.2 | 3.2 |
| SEM | 0.8 | 0.8 |

**Table 2** Percentage of cell death in the fronto-parietal area of the long-term vitamin E supplement group. All data are expressed as percentage of dead cells of individual animal, mean percentage and standard error of the mean of each group. Differences between groups are identified by using unpaired t-test and the level of significance of the difference is * P< 0.05.

| No. | Group1 | Group 2 |
|---|---|---|
| 1 | 45.85 | 39.41 |
| 2 | 44.91 | 39.80 |
| 3 | 36.37 | 27.65 |
| 4 | 37.55 | 36.17 |
| 5 | 39.71 | 25.33 |
| Mean | 40.88 | 33.67 |
| SD | 4.29 | 6.75 |
| SEM | 1.92 | 3.02 |

improved the severely expressed neurological signs, and promoted resynthesis of ATP after reperfusion for 3 hours[4]. In addition, α-tocopherol administered immediately after ischemia prevented ischemia-induced neuronal death evaluated after 7 days of survival[10]. Large dose of vitamin E supplement led to significant reduction of the infarct volume by 81% after 24 hours of right middle cerebral artery occlusion for two hours[11]. Long-term (13-16 weeks) vitamin E supplement significantly reduced (P=0.037) the infarct volume measured after 48 hours of survival in rats with permanent left middle cerebral artery occlusion[12]. However, recent evidence showed that short-term Supplement of alpha-tocopherol failed to reduce posttraumatic vasogenic brain edema[13]. Inconsistencies of the outcomes of treatment in different studies may be influenced by the ineffectiveness or inappropriateness of the method of drug evaluation and survival time after the insult. In these studies, the positive results were assessed by observing the recovery of brain electrical activity after reperfusion, measuring the infarct volume after 48 hours of survival, or measuring the oxidative products after reperfusion. In fact these indexes of experimental endpoint may not be sufficient to be used as measures of antiischemic efficacy. It is now accepted that neuropathologic evaluation performed with greater than 4 days survival is regarded as the gold standard for assessing cerebroprotection. Therefore, the present study may provide a more concrete evidence supporting the partial protective effect of vitamin E against acute ischemic brain damage.

Large-dosage antioxidant vitamin supplement is the most common self-prescribed vitamin in the elderly population. Their use and misuse are often unregulated with no concern on potential toxicity. This increase in vitamin use has, perhaps, been promoted by the belief that aging process is mediated, in part, by the production and accumulation of free radicals. Free radicals have the potential to affect protein structure and activity, alter gene expression and affect lipid metabolism[8]. Therefore, vitamin supplement may slow down the biomolecular damage associated with aging process and prevent diseases associated with aging. The present findings demonstrated more neuronal damages in older animals than in younger animals after acute ischemic attack. Furthermore, long-term vitamin E supplement could not prevent the old animals from acute ischemic brain damage. Hence, the present study agrees with several other studies demonstating that long-term vitamin E supplement neither delay aging in healthy elderly people, nor influence the outcome damage result-ing from transient ischemic attack. Therefore, vitamin E supplement should not be recommended in healthy elderly population. It should be reserved for individuals with documented deficiency or who are at risk only.

## REFERENCES


1. Florence T. The role of free radicals in disease. *Aust N Z J Ophthalmol* 1995; 23: 3-7.
2. Kirsch J, Helfaer M, Lange D, Traystman R. Evidence for free radical mechanisms of brain injury resulting from ischemia/reperfusion-induced events. *J Neurotrauma* 1992; 9 Suppl 1: S157-163.
3. Siesjo B. Cell damage in the brain: A speculative synthesis. *J Cereb Blood Flow Metabol* 1981; 1: 155-185.
4. Yamamoto M, Shima T, Uozumi T, et al. A possible role of lipid peroxidation in cellular damages caused by cerebral ischemia and the protective effect of alpha-tocopherol administration. *Stroke* 1983; 14: 977-982.
5. Floyd R. Role of oxygen free radicals in carcinogenesis and brain ischemia. *FASEB J* 1990; 4: 2587-2597.
6. Chanarat N. Role of lipid peroxidation and antioxidants in aging process and thalassemia. *Kitasato Arch Exp Med* 1992; 65: 245-249.
7. Sies H, Stahl W, Sundquist A. Antioxidant functions of vitamins: Vitamins E and C, β-carotene, and other carotenoids. *Ann N Y Acad Sci* 1992; 669: 7-20.
8. Thurman J, Mooradian A. Vitamin supplementation therapy in the elderly. *Drug Aging* 1997; 11: 433-449.
9. Fujimoto S, Mizoi K, Yoshimoto T, Suzuki J. The protective effect of vitamin E on cerebral ischemia. *Surg Neurol* 1984; 22: 449-454.

10. Hara H, Kato H, Kogure K. Protective effect of α-tocopherol on ischemic neuronal damage in the gerbil hippocampus. *Brain Res* 1990; 510: 2: 335-338.
11. Stohrer M, Eichinger A, Schlachter M, Stangassinger M. Protective effect of vitamin E in a rat model of focal cerebral ischemia. *Z Naturforsch* 1998; 53: 273-278.
12. Van der Worp H, Bar P, Kappelle L, de Wildt D. Dietary vitamin E levels affect outcome of permanent focal cerebral ischemia in rats. *Stroke* 1998; 29: 1002-1005.
13. Stoffel M, Berger S, Staub F, Eriskat J, et al. The effect of dietary alpha-tocopherol on the experimental vasogenic brain edema. *J Neurotrauma* 1997; 14: 339-348.