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CHANGES OF THE SERUM LEVELS OF THE MDA AND GSH WITH THE TREATMENT OF ALFA TOCOPHEROL IN THE EXPERIMENTAL BRAIN INJURY

Neurosurgery

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ABSTRACT

Free oxygen radicals which are toxic to the central nervous system are called as neurotoxic agents. Oxidative stress is seen as a result of several pathophysiological events including subarachnoid hemorrhage, edema, ischemia, hypertension, inflammatory reaction and trauma, leading to the production of lipid peroxidation products. In the present study, the malondialdehyde (MDA) level which is a lipid peroxidation product induced by traumatic brain injury as well the reduced glutathione (GSH) levels and glutathione peroxidase (GPx) enzyme activity which are indicators of antioxidant defense system were measured. Moreover, neuroprotective effects of alpha – tocopherol were investigated.

A total of 28 male Wistar rats were used in the present study. First group of rats underwent craniotomy with no trauma and accepted as sham group. The second group underwent craniotomi followed by trauma. The third group received intraperitoneal (i.p) injection of vehicle 8 hours before the craniotomy and trauma. The fourth group treated with i.p. alpha-tocopherol 8 hours before the craniotomy and trauma. All animals were decapitated 12 hours after the end of the treatment.

In the trauma group, MDA levels were significantly higher than the sham and alpha-tocopherol groups ($p<0.05$). GSH levels were significantly higher in alpha-tocopherol and sham groups compared to the trauma+ vehicle group ($p<0.05$). However, there was no significant difference between the sham and alpha-tocopherol groups ($p>0.05$). Although mean GPx activity was slightly higher in sham and alpha-tocopherol groups, there was no statistical difference between the groups.

In conclusion, MDA levels increase and GSH levels decrease in traumatized brain tissue. The results of present study suggest that trauma induces peroxidative cell injury by increasing the oxidative stress. Alpha-tocopherol may have neuroprotective effect by preventing the oxidative stress and related peroxidation.

KEYWORDS:

alpha-tocopherol, head trauma, MDA levels, GSH levels

INTRODUCTION

The effects of traumatic brain injury are caused by sudden and irreversible mechanical events [6]. Head trauma results in two types of injuries as the primary cell damage caused by the damage induced by actual blow and the secondary cell damage [14,18,20]. Free oxygen radicals have been shown to play a key role in the primary and secondary damages resulting from the head trauma and are neurotoxic agents [11].

In the primary damage resulting from head trauma, regional and diffuse damage occurs in the axons of nerve fibers in subcortical white matter in cerebral hemispheres and brain stem [22]. This primary damage leads to the formation of oxygen-induced free radicals and lipid peroxidation products, the release of excitatory glutamate and aspartate and the entry of calcium into the cell, causing the secondary cell damage by impairing the cell membrane permeability [4,6,17]. These reactive oxygen radicals are also associated with ischemia, subarachnoid hemorrhage and cerebral edema [9,12].

As a result of central nervous system cell damage, the cholesterol, gang lioside and alpha-tocopherol present in the membrane show structural and functional loss, initiating the peroxidative hydrolysis and inducing formation of free oxygen radicals [10,23].

There are several antioxidant defense systems including catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) protecting the cells from oxidative damage at the cellular level. Catalase is found in cerebral tissue in a low proportion, while SOD and GPx are found in a moderate proportion. Therefore, brain tissue is more susceptible to free oxygen radicals [12,21].

Although is not possible to avoid from the primary damage, the morbidity and mortality can be reduced by early recognition and treatment of secondary damage [8,21]. Recently, many treatment modalities have been tested with the aim of reducing the free oxygen radical-induced damage. For this purpose, phospholipase inhibitors, free radical scavengers, steroids, gangliosides and antioxidants were used. Pharmacological strategies are being tested to prevent these harmful effects [4,6,11].

Alpha tocopherol has been shown to have neuroprotective effects in the previous studies of traumatic brain injury. Recently, tocopherol and analogues have been suggested as an alternative treatment method for ischemic neuronal damage [11].

In the present study, the possible neuroprotective effect of alpha-tocop herol was investigated against the lipid peroxidation induced by free oxygen radicals associated wit traumatic brain injury. Open head injury model was induced in rats by using the modified Feeney eight-drop method and lipid peroxidation end-product, the MDA level as well as indicators of antioxidant defense systems, the GSH level and GPx activity wee measured biochemically.

MATERIALS AND METHOD

A total of 28 Wistar male rats weighing 270-330 g were used in the present study. Rats were obtained from TICAM animal laboratory of our faculty. Rats having free access to food and water were divided into groups of 7 animals in each, except for the first group consisting of 6 animals. Vehicle and alpha-tocopherol groups received i.p. injection of vehicle and alpha-tocopherol 8 hours before the surgery and trauma. The general anesthesia was induced by 50 - 60 mg/kg ketamine hydrochloride and 10 - 12 mg/kg xylazine hydrochloride injections. Moreover, 0.1 mg/kg atropine sulfate was given intraperitoneally in order to inhibit the secretions. After anesthesia, rats were intubated endotracheally by 6F angiocatheter. All animals were ventilated by 70% O2 and 30% athmospheric air with maintaining the tidal volume to be 2 mL.

Following the ventilation, the blood withdrawn from heart was used

for the measurement of arterial blood gas with maintaining the respiratory rate to a $PaCO_2$ of above 35 – 45 mm Hg and PaO_2 of above 100 mmHg. Body temperature was measured by using a rectal probe and maintained at 36,5 – 37,5 C during the surgical procedures. All surgical procedures were performed by using sterile surgical instruments, betadine, sterile gloves and mask.

Open brain damage in rats was induced by using the modified Feeney weight drop method [1]. The head was fixed with screws from both sides of the head in prone position. The target area was cleaned. Under sterile conditions, scalp was opened with a vertical incision. Then, skin was spread to both sides. The periosteal tissue was peeled off and a 1x0.5 cm craniectomy was performed on right parietal bone by the guidance of sagittal and coronal sutures and with using a high-speed dental drill and thin pin clamp. Dura was kept from the injury during the craniectomy procedure. A semi-translucent plastic tube 12.5-cm in length and 3.5-mm in inner diameter was placed to the craniectomy field with being perpendicular to the dura. Through this guide tube, a brass rod 15.2-cm in length, 3.5-cm in diameter and weighing 10 grams and being perpendicular to the craniectomy field, which was attached to an electrical relay at the top, was dropped onto the craniectomy field from a height of 5 cm and by switching off the circuit (The severity of the trauma = weight x height) and lesion was generated. Animals were re-anesthetized 12 hours after the trauma. Thoracotomy was performed in the supine position. Right heart auricula was removed. Left ventricle perfusion was performed by infusing 100 cc of saline under a pressure of 10 cm H_2O. Brain tissue was cleared from the blood. Then, animals were decapitated and brain tissue was removed from cranium. The contused area was seen in the right parietal cortex (Figure 1). Right hemisphere cortex was dissected from amygdala and hippocampal gyrus and frozen in liquid nitrogen and stored at – 70C.

The first group of animals (sham) underwent craniectomy with no trauma. The second group (trauma group) underwent craniectomy followed by trauma. This group was used to show the free oxygen radicals and lipid peroxidation products formed secondary to the trauma. The third group (vehicle group) received intraperitoneal sesame oil 8 hours before the trauma and used to evaluate the effects of vehicle. The last group of animals (treatment group) received intraperitoneal alpha tocopherol (vitamin E).

Because alpha-tocopherol needs a given period to enter the brain cell membrane, it was injected 8 hours before the trauma [11].

Biochemical measurements in the brain tissue

Reduced glutathione level was measured spectrophotometrically with using the Beutler method [1]. Brain tissue MDA level was determined by the method of Okhawa et al [16]. Brain GPx activity was measured by the method of Beutler [2].

Histopathological Evaluation

Histopathological procedure was performed with with hemotoxylin-eosine (HE) staining. For histological examination, all tissue samples were fixed at formaldehyde solution. Sections stained by HE were examined by an histologist experienced in its field under optical

Microscope (10x and 40x). Vacuolosis, cell deformation and necrosis were analyzed for histopathological changes.(Figure 2)

STATICAL RESULTS

Mean arterial blood gas values before the trauma are given in Table 1. Respiratory rate was set to maintain PCO_2 between 35 and 45 mmHg and PO_2 above 100 mmHg.

MDA levels in the brain tissue of rats decapitated 12 hours after the trauma are given in Table 2. The statistical analysis of MDA levels by using one-way variance analysis (Tukey – B) revealed significantly higher MDA levels in trauma group compared to the sham and alpha-tocopherol groups ($p<0.05$). Mean MDA level was slightly higher in the vehicle group compared to the trauma group with no statistical difference ($p>0.05$). Similarly, mean MDA level was slightly lower in the alpha-tocopherol group compared to the sham group with no statistical difference ($p>0.05$).

Brain GSH levels of the animals are shown in Table 2. GSH levels were significantly lower in trauma and vehicle groups compared to the sham and alpha-tocopherol groups ($p<0.05$). There was no significant difference between sham and alpha-tocopherol groups ($p>0.05$).

GPx activity measured in the present study is shown in Table 2. GPx activity was slightly higher in the sham and alpha-tocopherol groups with no statistical difference between the groups.

Three groups evaluated in histopathologic examination. In the trauma group, severe hemorrhage, necrosis and anormal shaped cell structures was observed (Figure 2).

DISCUSSION

When the oxygen concentration in the environment is above the normal, it results in toxic effects in aerobic organisms. As first described in 1954 by Gershman and Gilbert, this toxicity is caused by the effect of oxygen-induced free oxygen radicals instead of the oxygen itself [5,12].

Free oxygen radicals including superoxyde radicals, hydrogen peroxide and hydroxyl radicals are toxic to central nervous system (CNS). In other words, they are neurotoxic [10]. Neuronal damage seen after the head trauma includes both the effects of primary and secondary mechanisms. Recent studies have demonstrated increased neuronal degeneration caused by lipid peroxidation induced by the free oxygen radicals formed in the damaged brain tissue [13,21].

At the level of cellular membrane, lipid peroxidation is a chain reaction destroying the polyunsaturated fatty acids of membrane phospholipids. Brain tissue is particularly susceptible to the lipid peroxidation due to its high amounts of O_2 consumption. This lipid peroxidation has been shown to play a key role both in the primary and secondary stages of the brain injury [11,22]. Increased free oxygen radicals after the brain injury lead to loss of microvascular autoregulation, ischemia, membrane phospholipid peroxidation and a high amount of Ca+2 accumulation, all of which are considered to play a major role in post-traumatic cell injury and death. In addition, brain tissue contains a high amount of Fe+3 and Cu+2, which are the agents facilitating the formation of free oxygen radicals [21].

Present study evaluated the levels of lipid peroxidation product MDA and the antioxidant defense mechanisms GSH and GPx in posttraumatic brain tissue with the aim of investigating the possible beneficial effects of alpha-tocopherol. The modified Feeney method used in the present study is a cheap and easily applicable method resembling the pathophysiological changes in the brain injury [1].

Similar to the study by Kantos and Wei, we also created the lesion by leaving the dura intact with an aim of preventing the brain tissue laceration. If the lesion is created by the laceration of brain tissue, MDA levels will not be measured accurately. Because, MDA level is measured by TBA method, which may reveal incorrect results in a lacerated brain due to the light absorption by hemoglobin released from the brain tissue (9,12). TBA interactive substrate measurement method is the commonly used measurement method for in vivo produced free oxygen radicals. Although TBA is not specific to the MDA measurement, it is considered to be reliable indicator for lipid peroxidation [11].

In 1972, Ortega has suggested a pharmacological rationale for the treatment of head trauma with reporting that destruction in the brain tissue results mainly from the free oxygen radicals and that the main step in this process is increased vascular permeability which is caused by free oxygen radicals. Accordingly, Long et al. have also suggested that antioxidant agents may be used for the treatment of brain edema in the posttraumatic period [15,19,21]. The first tested agent for this purpose is the endogenous SOD enzyme, which has been shown to fail to decrease the free oxygen radical levels adequately [3,11].

Alpha-tocopherol is a lipid-soluble, effective antioxidant agent. In 1984, Buthon et al. have suggested that alpha-tocopherol is a potent chain-breaking agent. Peroxyl and alloxyl radicals formed by lipid peroxidation reacts with the alpha-tocopherol instead of this fatty acid side-chain, inhibiting he reaction. Ultimately, membrane stability and permeability are maintained [19,23]. Alpha-tocopherol is an antioxidant agent normally found in bio-membranes and plasma and can modulate the membrane permeability and stability. Ishii et al. have developed a compress brain edema model in order to investigate the clinical status in the epidural hematoma. In the studies by Yoshida et al. in 1983 and 1985 using this experimental model, the edema formation and water-Na+ changes were investigated 24 hours after the epidural compression in rats fed with vitamin E-poor, normal and vitamin E-

rich food. The authors have reported increased edema formation and water- Na+ uptake in rats fed with vitamin E-poor food, while decreased edema formation and water- Na+ uptake in rats fed with vitamin E-rich food[7,10,23,24].

In a similar study by Inci et al., mild- and severe-trauma groups were studied in terms of potential beneficial effects of 100 mg/kg i.p. alpha-tocopherol injected before the trauma. Lipid peroxidation was found to be significantly lower in alpha-tocopherol-treated rats with mild trauma compared to non-treated animals in the mild trauma group. Animals in the severe trauma groups also showed a similar significant difference [11]. In 1995, Grisar has demonstrated alpha-tocopherol and its analogues to prevent the in vivo and in vitro lipid peroxidation, protecting the animals form the damage caused by CNS trauma [7,19]. In the present study, alpha-tocopherol was intraperitoneally injected to the rats 8 hours before the trauma to allow adequate amount of the alpha-tocopherol to reach the cerebral cell membrane. Rats were decapitated 12 hours after the trauma to allow adequate edema formation and MDA, GSH and GPx levels were measured in brain homogenates from the rats with contusion performed by modified Feeney method.

In alpha-tocopherol-treated trauma group, lipid peroxidation product MDA was significantly lower compared to the untreated animals with a statistical difference between these two groups ($p<0.05$). On the other hand, GSH level was significantly higher in sham group compared to the trauma and vehicle group ($p<0.05$). There was also a slight difference in GSH level between sham and alpha-tocopherol groups with no statistical significance. It can be suggested that alpha-tocopherol hs beneficial effects on antioxidant defense systems by preventing the lipid peroxidation. Accordingly, GPx levels were different between vehicle and trauma group and sham and alpha-tocopherol groups but with no statistical significance.

Results of the present study suggest that alpha-tocopherol decreases the lipid peroxidation and support the previous literature data [11]. In the future, it is possible that alpha-tocopherol and other antioxidants will be used for their not only neuroprotective effects but also for their therapeutic potentials.

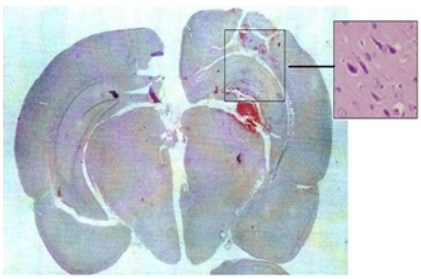

FIGURE LEGENDS

Figure 1: The contused area was seen in the right parietal cortex after craniotomy

Figure 2: After traumatic brain injury, in the coronal sections structural changes and necrosis observed in light microscobe (10x and 40 x).

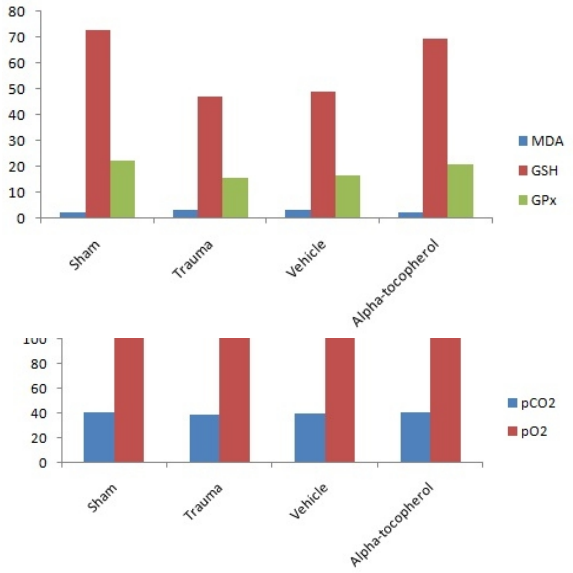

TABLE LEGENDS

Table 1: Blood gas levels in sham, trauma, vehicle and treatment groups (mmHg)

Table 2: MDA, GSH and GPx levels in sham, trauma, vehicle and treatment groups(nmol / mg protein)

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