LIPID PEROXIDATION AFTER INTRACORTICAL INJECTION OF FERRIC CHLORIDE INCREASES THE INCIDENCE OF SEIZURES IN YOUNG RATS

Nyoman Golden[1]✉, Nyoman Agus Bagiada[2], Sajid Darmadipura[2]

[1]School for Graduate Study, Udayana University
[2]Faculty of Medicine, Udayana University
E-mail: nyoman_golden@yahoo.com

ABSTRACT

Clinical studies have shown that the incidence of early posttraumatic seizures is higher in children than in adults and it has been proposed that iron-induced lipid peroxidation has an important role in the development of epileptogenic foci. In this study, we examined some of the hypothesized reasons for the difference in the incidence of early posttraumatic seizures between young and adult rats. Twelve young and twelve adult rats were randomized into 4 groups. Group 1 and 2 were control groups, each comprising of 6 young rats and 6 adult rats respectively and were given intracortical injections of normal saline. Group 3 and 4 were injury groups, again comprising 6 young rats and 6 adult rats respectively and were given intracortical injections of $FeCl_3$. All rats were observed for 6 hours post injection for the occurrence of seizures and were then killed. The injected hemispheres were extirpated and tested for malondialdehyde (MDA) level and superoxide dismutase (SOD) activity as indices of oxidative damage. Results showed that seizures were observed only in Group 3. Increased MDA level and decreased SOD activity were observed in Group 3 (ANOVA, $p<0.001$). Increased MDA levels and decreased SOD activity were significantly higher in rats with seizures (Group 3) than in those without seizures (independent *t*-test, $p<0.001$). We conclude was that different levels of lipid peroxidation induced by intracortical ferric chloride injection may account for the different seizure incidence between young and adult rats.

Key words: early posttraumatic seizures, lipid peroxidation, epileptogenic foci, malondialdehyde, superoxide dismutase

INTRODUCTION

Clinical studies have consistently found that the incidence of early posttraumatic seizures in children is higher than in adults, although the incidence varies widely (Annegers *et al.*, 1980; Desai *et al.*, 1983; De Santis *et al.*, 1992). Head injury, with extravasation of red blood cells followed by hemolysis and deposition of iron compounds within the brain tissue, is associated with development of posttraumatic seizures (Payan *et al.*, 1970; Willmore *et al.*, 1983). The same thing happens when iron salt or heme is injected intra ortically in the rat brain, which can induce seizures and epileptic discharges

(Willmore *et al.,* 1978; Kabuto *et al.,* 1998). It has been proposed that oxygen free radicals generated by iron-mediated reactions in the brain can peroxidize lipid compounds in the neuronal membrane producing epileptogenic foci (Willmore *et al.,* 1978; Willmore and Rubin, 1982; Triggs and Willmore, 1984).

The activities of antioxidant enzy mes such as SOD, catalase and glu tathione peroxidase (GPx) in the immature brain are low (Mavelli *et al.,* 1982; Aspberg and Tottmar, 1992; Hussain *et al.,* 1995; Dringen, 2000). The low activities of these antioxidants make the immature brain more suscep tible to oxidative stress, which is shown by higher lipid peroxidation (Koudelova and Mourek, 1994; Ferriero, 2001; Buonocore *et al.,* 2002; McLean and Ferriero, 2004; Vannucci and Hagberg, 2004).

MDA is a secondary product of lipid peroxidation and has been used to evaluate oxidative stress (Kabuto *et al.,* 1998; Suzer *et al.,* 2000; Halliwell and Whiteman, 2004), while antioxidant enzymes that have been used to evaluate oxidative stress include SOD and GPx (McMichael, 2004; Bayir *et al.,* 2002; Fan *et al.,* 2003). Under conditions of oxidative stress there is an increase in the MDA level and a decrease in SOD and GPx activities (Bayir *et al.,* 2002; Ozdemir *et al.,* 2005).

In this study we test the hypothesis that the higher levels of lipid peroxidation in immature brain and the role of lipid peroxidation in the development of epileptogenic foci may account for the difference in seizure incidence between children and adults.

MATERIALS AND METHODS

Twelve young Wistar rats (14 days old) and twelve adult Wistar rats (90 days old) (Kaudelova and Mourek, 1994) were used in this experimental study. The animals were placed in individual cages under controlled conditions (12 hour day and night cycle in 26^{o}C room temperature) with free access to food and water. All animals were anesthetized with intramuscular injection of ketamine (50 mg/kg). Epileptic foci were induced by injecting $FeCl_3$ in the left sensory-motor cortex (Kabuto *et al.,* 1998) using the procedure described by Willmore *et al.*1978. The rats were placed in the stereotaxic device and burr holes of 2 mm in diameter was made in the cranial bone at a point 1 mm posterior and 2 mm lateral to the bregma. A needle (25G) of a micro-syringe was inserted through the hole with the tip inserted into the cortex, about 1.6 mm deep in the exposed dura mater (Kabuto *et al.,* 1998). The rats were randomized and divided into 4 groups. Group 1 and 2, the control groups, comprising 6 young rats and 6 adult rats respectively, were injected with saline. Group 3 and 4, again comprising 6 young rats and 6 adult rats respectively, the injury groups were injected with 5µL of freshly prepared aqueous solution of 50 mM $FeCl_3$, (Sigma Chemical Co., St. Louis, MO, USA). All rats were observed for 6 hours post injection for seizure events after which they were killed and decapitated. Their left hemispheres were extirpated and tested for MDA levels and SOD activities.

All data were expressed as mean ± standard deviation. One way ANOVA followed by LSD post hoc-test were used for multiple comparisons.

Comparisons between two groups were analyzed by independent *t*-test. A *p* value of <0.05 was considered signi ficant.

RESULTS

Seizures were observed only among young rats given $FeCl_3$ intracortical injection (Group 3). Table 1 shows the MDA level and SOD activity in the rat brains in each group. The MDA level was significantly higher in Group 3 than in the other groups (ANOVA, $p<0.001$). The SOD activity was significantly lower in Group 3 than in the other groups (ANOVA, $p< 0.001$). Table 2 shows that the MDA level in rats with seizures was significantly higher than in those without seizures (indepen dent *t*-test, $p< 0.001$). The SOD activity in rats with seizures was significantly lower than in rats without seizures (independent *t*-test, $p<0.001$).

DISCUSSION

It has been proposed that intra cortical iron injection causes seizures and epileptiform discharges on EEG (Willmore *et al.*, 1978; Kabuto *et al.*, 1998; Suzer *et al.*, 2000). The histological appearance of the epileptic model is similar to that observed in human posttraumatic epileptogenic foci (Willmore and Rubin, 1982).

Table 1 Malondialdehyde (MDA) levels and superoxide dismutase (SOD) activities

Groups	MDA (μg/100mg wet tissue)	SOD (U/100mg wet tissue)
Young rats/control (group 1, *n*=6)	0.14050±0.020491	9.76333±1.016792
Adult rats/control (group 2, *n*=6)	0.14067±0.008116	9.37500±0.745191
Young rats/injury (group 3, *n*=6)	0.23183±0.025686*	2.95833±0.769192*
Adult rats/injury (group 4, *n*=6	0.11333±0.030690	10.19000±0.377412

Values are expressed as means±standard deviation
*$P<0.001$ compared with all groups

Iron facilitates the production of free radicals either via the Fenton or Haber-Weiss reaction. Free iron catalyzes the conversion of the radical superoxide to highly reactive hydroxyl radicals, a compound that can attack all molecules in living cells (Graf *et al.*, 1984; Brau ghler, 1986; Minotti and Aust, 1987; Eberhardt, 2001). Studies using sei zure models have been conducted in adult rats with a dosage of 100 mM iron salt (Willmore *et al.*, 1978; Kabuto *et al.*, 1998; Willmore *et al.*, 1986; Yokoi *et al.*, 1995; Suzer *et al.*, 2000). Willmore *et al.* 1986 reported that injection of 25 and 50 mM iron salt did not cause seizures. Convulsive seizures or generalized epileptiform discharges occurred with 100 mM iron salt and the product of lipid peroxydation in rats with seizures was higher than in rats without seizures. We used the dosage of 50 mM for all groups of rats, with the assumption that in the clinical situation, the same degree of head injury gives greater chance for early posttraumatic seizures among children

Table 2 MDA level and SOD activity of seizure rats and non-seizures rats

	Seizure rats	Non-seizure rats	P value
MDA (μg/100mg wt net)	0.23183±0.025686	0.13150±0.024385	<0.001
SOD (U/100 mg wt net)	2.95833±0.769192	9.77611±0.791583	<0.001

Values are expressed as means±standard deviation

MDA is formed from polyunsaturated fatty acid breakdown and is the main secondary product of lipid peroxidation. MDA provides a simple way to determine the level of lipid peroxidation (Kabuto *et al.,* 1998; Willmore *et al.,* 1986; Suzer *et al.,* 2000). SOD, an antioxidant enzyme that converts the superoxide radical to hydrogen peroxide can also be used to measure the level of oxidative stress ((McMichael, 2004).

The activities of endogenous enzymatic antioxidants such as SOD, catalase and GPx increase with age and are lower in the immature brain (Mavelli *et al.,* 1982; Aspberg and Tottmar, 1992; Hussain *et al.,* 1995; Dringen, 2000). Kaudelova and Maurek 1994 reported that MDA levels in immature brains are higher than in mature brains during oxidative stress. The results of our study also showed that the rats' immature brain tissue was more susceptible to oxidative stress induced by intracortical iron salt injection. This was proven by the high levels of MDA and the low SOD activities.

Free radicals and other lipid peroxidation products can damage the SH group of excitatory amino acid transporter (EAAT) (Volterra *et al.,* 1994) and down regulate its production (Doi *et al.,* 2005). This condition reduces the ability of EAAT to uptake glutamate. Glutamate is an important excitatory neurotransmitter in the brain. Lipid peroxidation decreases the release of GABA (Zhang *et al.,* 1989), which is a well-known inhibitory neurotrans mitter. Thus lipid peroxydation causes disturbance of extracellular neuro transmitter regulation. All the above conditions increase synaptic transmis sion of excitatory glutaminergic and neurotoxic glutamate, which closely correlated to seizures pathophysiology (Ueda *et al.,* 2003).

The role of lipid peroxidation in the development of seizures was also observed in surgically resected epileptic foci of human brain tissues. The examination of epileptic foci in patients with intractable seizures revealed higher levels of MDA and lower SOD activities compared to the brain tissue surrounding the epileptic focus (Mori *et al.,* 1990). Our results also support the role of lipid peroxidation in seizures. We also observed that the level of peroxidation in rats with seizures was higher than in those without.

Novelty

This is a preliminary study showing that different level of lipid peroxidation is associated with different ces in seizures incidence in immature and mature brains.

CONCLUSION

The level of lipid peroxidation in young rats after intracortical injection of 5 μL $FeCl_3$ 50 mM was significantly higher compared to other groups.

The level of lipid peroxidation in rats with seizure was significantly higher compared to those without seizure; on the contrary, the activity of SOD in rats with seizure was significantly lower as compared to those without seizure.

This study revealed that the level of lipid peroxidation after intra cortical injection of $FeCl_3$ might account for the difference seizures incidence between young and adult rats.

SUGGESTION

It is suggested that a similar study be conducted in the clinical setting by use of cerebrospinal fluid to evaluate the oxidative condition of brain tissue.

Further work should be carried out to develop exogenous antioxidants to suppress the lipid peroxidation in immature brain. This is intended as a possible new approach to control seizure in view of the present ineffective conventional antiepileptic drugs such as phynitoin and phenobarbital, which are still being used in children (Young *et al.,* 1983).

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