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GLOBAL İSKEMİ VE REPERFÜZYONDA BEYİN GSH-Px AKTİVİTESİ: GLUTAMAT SALINIM İNHİBİSYONUNUN ETKİSİ

BRAIN GSH-Px ACTIVITY IN GLOBAL ISCHEMIA AND REPERFUSION: THE EFFECT OF GLUTAMATE RELEASE INHIBITION

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ÖZET: Glutatyon Peroksidaz (GSH-Px) enzim aktivitesi geçici olarak hipoperfüzyona tabi tutulan ratların değişik beyin bölgelerinde ölçülmüştür. Serebral hipoperfüzyon, bilateral Arteria Carotis Communis'in 30 dakika geçici oklüzyonu ve hipotansiyon indüksiyonunu takiben 60 dakika reperfüzyon uygulanarak gerçekleştirilmiştir. Glutamat salınım inhibisyonu için, iskemi oluşturulmasından hemen sonra intraperitoneal Lamotrigine (LTG) verilmiştir. Kortikal, subkortikal ve serebellar bölge GSH-Px aktivitesi tayin edilmiştir. İskemi uygulanan ratlarda, GSH-Px aktivitesi özellikle subkortikal ve serebellar bölgelerde, kontrol ratlarına göre daha düşük bulunmuştur ($p<0.001$). Reperfüzyon uygulanan ratlarda, tüm beyin bölgelerinde GSH-Px aktivitesindeki düşüş devam etmiştir ($p<0.001$). Glutamat salınım inhibisyonu, tüm beyin bölgelerinde GSH-Px aktivitesini önemli ölçüde arttırmış, hatta kortikal ve serebellar bölgede kontrol değerlerinin üzerinde artış sağlamıştır ($p<0.05$). İskemi ve reperfüzyonda GSH-Px aktivitesindeki düşüş, tiyol gruplarının okside olmasına ve dolayısıyla enzimin substratı olan redükte glutatyonun azalarak, enzim aktivitesinde düşüşe neden olabileceği şeklinde açıklanabilir. LTG tedavisi ile tiyol grupları oksidasyonunun azalarak dolaylı olarak GSH-Px enzim aktivitesinde artışa yol açması gözlenmiştir.
[Anahtar Kelimeler: GSH-Px, Serebral İskemi, Reperfüzyon, Lamotrigine]

ABSTRACT: Glutathione Peroxidase (GSH-Px) activity was determined in different brain regions of rats after transient hypoperfusion. Cerebral hypoperfusion was induced by the occlusion of the bilateral Common Carotid Arteries and hypotension induction for 30 min and followed by 1-hour reperfusion period. Lamotrigine (LTG), a glutamate release inhibitor was given intraperitoneally just after the induction of ischemia. GSH-Px activity was determined in cortical, subcortical and cerebellar regions. Subcortical and cerebellar GSH-Px activities were found to be lower than those in control rats ($p<0.001$). The decrease in GSH-Px activity in all brain regions was persisted during reperfusion ($p<0.001$). Glutamate release inhibition significantly increased the GSH-Px activities in all brain regions even above the control levels in cortical and cerebellar regions ($p<0.05$). The decrease in GSH-Px activity during ischemia and reperfusion could be attributed to the fact of oxidized thiol groups and consequently decreased levels of enzyme substrate, reduced glutathione (GSH). Increased GSH-Px activity could be explained by reduction in thiol oxidation in treatment group.
[Key Words: GSH-Px, Cerebral ischemia,reperfusion, Lamotrigine]

INTRODUCTION

Cerebral ischemia is a common neurologic disorder classified as either focal or global ischemia (1). The prototype of global brain ischemia and reperfusion is cardiac arrest and cardiopulmonary resuscitation (2).

Since central nervous system (CNS) neurones are vulnerable to ischemia and reperfusion injury; two major hypothesis are of great importance: excitotoxic neurotransmitter hypothesis which is related with the events during ischemia and free radical hypothesis which suggests radical induced damage during reperfusion. This vulnerability is due to low activity of Glutathione Peroxidase (GSH-Px) (3) and high endogen iron content of brain (4).

In this study, we assessed the changes in GSH-Px activities in different brain regions in rats proned to global cerebral ischemia and reperfusion injury. The effects of glutamate release inhibition on GSH-Px activity were also studied.

MATERIALS AND METHODS

Thirty two male Swiss-Albino rats weighing 190-270 g were assigned into one of four groups: sham operated controls, ischemia, reperfusion and treatment groups. Each groups contains 8 animals. Ischemia was induced for 30 min by the occlusion of the bilateral Common Carotid Arteries and the induction of hypotension by drawing blood from tail vein. In reperfusion group, ischemia period was followed by 1-hour reoxygenation. Lamotrigine (LTG), a glutamate release inhibitor, was administered intraperitoneally (20 mg/kg) just after the induction of ischemia and hypotension. The ischemia and reperfusion periods were followed similarly in treatment group.

The brains were removed by decapitating the rats at the end of the each surgical procedure and immediatelly divided into cortical, subcortical and cerebellar tissues. The homogenization of the tissues were performed in an ice-containing medium in 3 ml of cold potassium phosphate buffer (100 mmol/L, pH=7,5). Following homogenization, samples were centrifuged for 20 min (14.000 rpm, +4°C). The supernatants were immediatelly removed and used for biochemical analysis. GSH-Px activities were determined according to the modification of the technique described by Paglia and Valentine (5). Protein levels of the samples were determined by the method of Lowry et al.(6).

Data are expressed as mean±SD. One-way analysis of variance (ANOVA) was used to determine the significance of any difference between the groups. A two-tailed paired Student's *t* test was used to determine the differences between different brain regions.

RESULTS

Brain tissue GSH-Px levels are shown in Table I, Figure I. Subcortical and cerebellar tissue GSH-Px activities in ischemia group were found to be significantly lower than those in controls ($p<0.001$, $p<0.05$ respectivelly). In reperfusion group, GSH-Px activities in all brain regions dropped significantly than those in controls and ischemic animals ($p<0.001$). In treatment group, LTG reversed the decrease in GSH-Px activities in all brain regions when compared to ischemia and reperfusion group ($p<0.001$). LTG treatment increased the GSH-Px activities especially in cortical and subcortical tissues when compared to controls ($p<0.001$, $p<0.05$ respectivelly).

DISCUSSION

Cerebral ischemia is still the third major cause of death and one of the major cause of long-term disability in developed countries. Either focal or global ischemia is the major cause of cerebral ischemia (1).Brain which is the only 2% of body weight, receives 20% of cardiac output and fully is dependent of complete glucose oxidation. The loss of energy production and the alterations in ionic environment cause extracellular accumulation of glutamate, an excitotoxic amino acid. Ten-min ischemia causes 5-fold increase whereas

20-min ischemia causes 10- to 15- fold increase in glutamate concentration (7,8).

GSH-Px (EC 1.11.1.9) is a selenoprotein and plays an important role against oxidative damage by catalyzing the reduction of hydroperoxides by using reduced glutathione (GSH) as the reducing substrate. It is evident that hydroxyl radicals were produced during anoxia/reoxygenation of isolated brain microvessels. The hydroxyl radicals were suppressed if catalase and superoxide dismutase (SOD) were present. It is interpreted that; anoxia/reoxygenation causes superoxide radical and hydrogen peroxide formation on the surface of microvasculature cells. They all re-enter the cells and cause hydroxyl radicals (9).

There are numerious studies indicating the role of oxidative stress in the neuronal degeneration and cell death that occur during ischemia and reperfusion injury. It has been shown that GSH-Px overexpressed mice displayed decreased infarct size and better neurologic outcome and decreased levels of 4-hydroxynonenal modified proteosome subunits after ischemia-reperfusion injury compared with GSH-Px devoided mice (10). Transgenic mice overexpressing the intracellular form of GSH-Px showed decreased infarct size after focal cerebral ischemia and reperfusion injury (11). Previously it was reported that SOD, GSH-Px activities were found to be decreased in reperfusion injury compared to non-ischemic and ischemic animals. Whereas the catalase activity was found to be increased (12). Besides this, no changes were observed in SOD, GSH-Px, glutathione reductase and glucose-6-phosphate dehydrogenase activities in the brains of newborn piglets after ischemia and reperfusion in another study (13).

Different chemical agents were tested to detect the protective efficacy on different antioxidant enzymes in ischemia reperfusion models. Ebselen, which is known to have GSH-Px-like activity, reduced the lactate and purine catabolites in transient ischemia (14). It was also reported that high-dose Ebselen treatment significantly decreased the infarct volume in focal cerebral ischemia (15). It was found that Schisanhenol and Schizandrin induced increase of cytosolic GSH-Px activities in brain in mice in reperfusion period (16).

LTG is an antiepileptic agent which blocks voltage-dependent sodium channels and prevents excitatory neurotransmitter release. It is effective against partial and generalized tonic-clonic seizures (17). Glutamate-induced neurotoxicity underlie the cell injury and necrosis in cerebral ischemic injury is inhibited by blocking excitatory neurotransmitter release (18,19). LTG shows cerebro-protection against focal cerebral ischemia, reduce infarct volume after permanent middle cerebral artery occlusion in the rat, an effect presumably attributable to suppression of excitotoxic glutamate release (20).

There are limited number of studies in which LTG has been used in global cerebral ischemia. It was reported that LTG treatment significantly protected hippocampal CA1 neurones (21,22). It was reported that LTG reversed the impaired neurobehavioral function after cerebral ischemia in rabbits (23). Previously we have shown that, LTG pre-and post-treatment during focal cerebral ischemia significantly decreased the brain nitric oxide (NO) and cyclic guanosine monuphosphate (cGMP) levels in rats (24).

In the present study, the effects of LTG on brain GSH-Px activities were evaluated. LTG reversed the decrease in GSH-Px activities in all brain regions when compared to reperfusion and ischemia group. Another important finding is the observation of increased GSH-Px activities in cortical and subcortical tissues in treatment group. The decrease in GSH-Px activities could be attributed to the loss of thiols especially in GSH, which is the substrate for GSH-Px, during ischemia and reperfusion (unpublished data). LTG treatment was

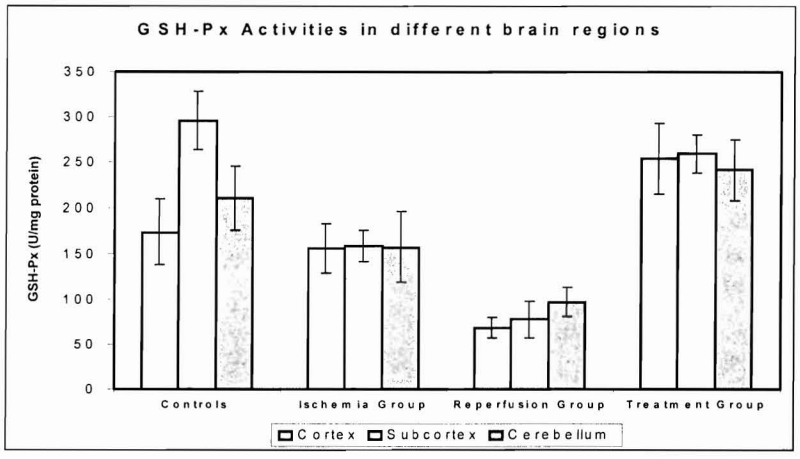
thought to be successfull in reversing the loss in thiol content and indirectly preserved the GSH-Px enzyme activity. This study seems to be unique identifying the effects of LTG on brain GSH-Px activities during global ischemia and reperfusion

Table 1: GSH-Px activities of different brain regions in various groups (Mean ± SD).

GSH-Px (U/mg protein)	Controls (n=8)	Ischemia Group (n=8)	Reperfusion Group (n=8)	Treatment Group (n=8)
Cortex	174.00±35.95	156.25±27.15	68.38±11.40 [a,c]	253.88±38.66 [a,c,e]
Subcortex	295.87±31.93	159.00±17.26 [a]	77.88±20.46 [a,c]	259.50±20.91 [b,c,e]
Cerebellum	211.00±34.42	157.50±38.58 [b]	97.13±16.50 [a,d]	241.38±33.35 [c,e]

[a]p<0.001 Significantly different from controls.
[b]p<0.05 Significantly different from controls.
[c]p<0.001 Significantly different from ischemic group.
[d]p<0.05 Significantly different from ischemic group.
[e]p<0.001 Significantly different from reperfusion group.

Figure 1. GSH-Px activities of different brain regions in various groups.



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