The effect of selective COX-2 inhibitor on blood glutamate in moderate traumatic brain injury

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Abstract

Head injury is the leading cause of death and disability in adolescence, children and the elderly. Post-traumatic brain damage is determined by combination of primary and secondary head injuries. Neuroinflammation is one mechanism of secondary brain injury. Selective cyclooxygenase (sCOX-2) inhibitors are drugs commonly used in treatment of postoperative pain but also possess an anti-inflammatory effect. The aim of this study is to determine the role of sCOX-2 inhibitors to inhibit the inflammatory processes in patients with head injury by measuring the glutamate levels.

This is a double blind randomized controlled study involving patients with moderate head injuries who underwent surgery at Dr. Hasan Sadikin Hospital Bandung since December 2013 until December 2015. After obtaining study approval from the Research Ethics Committees of School of Medicine Padjadjaran University/Dr. Hasan Sadikin Hospital, samples were clustered randomly into 5 groups: the control group, the COX2-group I (given sCOX-2 inhibitor once/day), the COX2-group II (given sCOX-2 inhibitor twice/day), the COX2-group III (given sCOX-2 inhibitor thrice/day), and the COX2-group IV(given sCOX-2 inhibitor four times/day), and each group consisted of 6 patients. All patients received standard therapy as recommended by Brain Trauma Foundation Guidelines 2007 as well as performed monitoring of blood pressure, pulse rate, respiratory rate, oxygen saturation, temperature and blood sugar during pre and postoperative stages. The data were analyzed using paired samples t-test and one-way Anova, which $p<0.05$ is considered as statistically significant.

Results showed that there was a significant reduction in glutamate level in COX2-group II with the p-value of 0.035.

The study concluded that sCOX-2 inhibitor has a brain protective effect by lowering the levels of glutamate as neuroinflammatory biomarkers in patients with head injury.

Key words: Glutamate, mild traumatic brain injury, neuroinflammation, selective COX-2 inhibitor.

Introduction

Approximately 2.5 million emergency department visits, hospitalizations, and deaths in the United States were due to traumatic brain injury (TBI) as estimated by the Centers for Disease Control and Prevention (CDC), either treated as isolated injury or in combination with other injury. Of those statistical report depicted in 2010, approximately 87% were treated in and released from emergency department, another 11% were hospitalized and discharged, and approximately 2% died. (1) The incidence of head injury at our hospital, Hasan Sadikin Hospital in Bandung, Indonesia, in 2010-2011 was approximately 300-425 cases per month and mostly were moderate head injuries.

Mortality rate in mild head injury was reported approximately 6.6% whereas moderate and severe head injuries were 15%. Moderate and severe head injuries posses the rate of disability and vegetative state 16-38% with the treatment cost about 60 billion US dollar. Mortality rate in severe head injury was reported about 39.8% of total 18,002 patients. (2-5)

The post-traumatic brain damage depends on the combination of primary head injury and secondary head injury. Primary head injury causes biomechanical effect of the forces applied to the skull and brain at the time of occurrence and is manifested within milliseconds, which until now there is no treatment for primary brain injury other than

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giving prevention to reduce the severity of injury. On the other hand, secondary brain injury developed at the later stage and during perioperative period after the primary brain injury. Secondary brain injury, specifically with complex cascade from biochemist and biomolecular, may develop neuroinflammation, brain edema, and delayed brain cell death. (2,3)

Pathophysiology of mild, moderate and severe head injuries have a similar way. The different categories of mild, moderate and severe brain injuries depend on Glasgow Coma Scale (GCS) score, the severity and the wide of the area of injury during first impact, and the following secondary brain injury. After head injury, patient may suffer hypoxia that will accelerate brain ischemia and reperfusion injury, which influences patient outcome after the injury. The management of head injury as pharmacologic brain protection with intravenous and inhalation anesthetics, lidocaine, mannitol, magnesium, erythropoietin, alpha-2 agonist dexmedetomidine, hypertonic natrium lactate, anti inflammatory drug cyclooxygenase (COX)-2 inhibitor, and corticosteroid. Some of these drugs are still ongoing researches. (5)

Several reports between 1977-2002 have reported that morbidity and mortality rates were still high in patients with managed intracranial pressure (ICP) and cerebral perfusion pressure (CPP), and therefore, the use of pharmacological brain protection is encouraged. Several pharmacological researches with the objective of knowing brain protector effect until now are still ongoing process, but currently there is no available drugs yet to prevent secondary brain injury applied as one strategy therapy for TBI. (2)

Glutamate is amino acid that is released after TBI. In the ischemic cerebral condition, there are increasing releases of neurotransmitter excitatory amino acid (EAA) glutamate and aspartate. There are 3 receptors from the neurotransmitter excitatory, which have been identified, i.e. N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), and metabotropic receptors. NMDA receptor mediates influx of natrium and calcium through membrane canal. Magnesium and dizocilipine maleate blocked NMDA receptor competitively. AMPA and metabotropic receptors mediate influx of natrium. Glutamate stimulates 3 receptors, but aspartate only affects NMDA receptor. Glutamate causes neuronal cell death through early and delayed mechanisms. (6-10)

In the early phase of neurotoxicity, glutamate will activate NMDA receptors, and cause natrium, chloride, and water enter the cell, and consequently, cellular edema, membrane cell lysis, and cell death develop. In the delayed phase of neurotoxicity (24-72 hours), NMDA receptors are activated and stimulate ischemic cycle, which initiate calcium entry into the cell. This situation will activate phospholipase, protease, and free fatty acids/FFAs activities, which further produce arachidonic acid and free radical, lipid peroxide, and then lead to the cell death. (10,11)

During several last periods, it was known that secondary brain injury releases cytokine proinflammatory, prostaglandin, chemokine, glutamate, free radical and lead to apoptotic cell death. Head injury causes inflammatory disease thus, logically, it is treated with anti-inflammatory drugs. Corticosteroid and non-steroidal anti-inflammatory drugs (NSAID) COX-1 inhibitor and COX-2 inhibitor have been well known as analgetic anti inflammation and have been used as selected drugs for postoperative pain. In 2004, there was a research on high dose corticosteroid (methylprednisolone) after significant head injury (CRASH) with around 20,000 head injury patients. The research has been stopped and failed because of increasing mortality rate up to 50% in 24 days. For this reason, the current guidelines avoid giving corticosteroid to head injury patients. (2,3,12-14)

Brain cell death after head injury is triggered through releasing glutamate and cytokine proinflammatory. Over expression COX-2 will have worst outcome, and so, inhibition of COX-2 will delay cell death and neuroinflammation and therefore, the hypothesis is defined by using NSAIDs with strong anti-inflammatory effect like COX-2 inhibitor for head injury patients. (15)

Hypothesis

Selective COX-2 inhibitor given intravenously has brain protective effect in patients with moderate head injury by decreasing glutamate level.

Subject and method

Experimental randomized controlled trial (RCT), double blind, 30 patients with moderate TBI underwent neurosurgery at Dr. Hasan Sadikin Hospital, Bandung with inclusion and exclusion criteria as listed below:

Inclusion criteria:

1. Man and woman 13-60 year-old.
2. Head injury with GCS 9-12, without other primary injuries.
3. All patients met requirements for evacuation intracranial hematoma (epidural hematoma, subdural hematoma, intracranial hematoma).

4. Head injury occured less than 24 hours.
5. Physical status ASA II.

Exclusion criteria:

1. History of using continuous NSAID during the last 30 days.
2. Unstable blood pressure (systolic blood pressure <90 mmHg).
3. Pregnant women or menstruation.

Drop out criteria:

1. Patient death before 3 days postoperative.
2. Length of surgery more than 4 hours.

Statistical measurement for general characteristic with one-way Anova, excludes sex variable with Chi Square. The significant difference is valid with $p<0.05$ and very significant difference is valid with $p<0.01$. Statistical analysis for glutamate level is using Paired t-test, and one-way Anova.

Research methodology

The research is initiated after obtaining approval from the Ethical Clearance of School of Medicine Padjadjaran University/Dr. Hasan Sadikin Hospital, Bandung. After informed consent has been cleared, patient's (with moderate head injury [GCS 9-12] without other primary injuries) heads were positioned up to 30^0, blood pressure, core temperature, blood sugar, and SpO2 were measured. Ten cc of venous blood was drawn for measuring glutamate level as basic data.

Samples were divided into four treatment groups COX2 and one control group. Treatment groups (COX2) were then classified into 4 sub groups: COX2-I, COX2-II, COX2-III, and COX2-IV (six patients each group), and COX-2 inhibitor 40 mg was given intravenously with the sequence orders as listed below:

1. Group COX2-I: COX-2 inhibitor was given once/day
2. Group COX2-II: COX-2 inhibitor was given twice/day
3. Group COX2-III: COX-2 inhibitor was given thrice/day
4. Group COX2-IV: COX-2 inhibitor was given 4 times/day
5. Group control: NaCl 0.9% was given before the induction of anesthesia

Intravenous induction was performed using propofol 2 mg/kgBW, vecuronium bromide 0.8 mg/kgBW, fentanyl 2 μg/kgBW, lidocaine 1.5 mg/kgBW and isoflurane 1.5 MAC with 6 L/minute oxygen. After the induction, intubation was performed using non-kinking endotracheal tube. Maintained anesthesia with isoflurane 1 MAC, oxygen 3 L/minute, air 2 L/minute, continuous propofol 0.5-1 mg/kgBW/h, and continuous vecuronium 0.1 mg/kgBW/h. Venous line was added with vein catheter no 18. Urinary catheter was applieda also. Ventilation was controlled during surgery. Mannitol was given 0.5 g/kgBW intravenously. All groups received postoperative analgetic metamizole 500 mg intravenously.

Varied among groups, COX2-II group received COX-2 inhibitor again every 12h, COX2-III group every 24h, and COX2-IV group every 36h after preinduction, whereas 2 cc NaCl 0.9% was given to control group. Ten cc of blood was taken every 6h after the last usage of COX-2 in subgroup I, II, III, and IV to measure glutamate levels. During blood taking, the blood pressure, core temperature, blood glucose, and SpO2 were measured.

Results

Table 1 describes general characteristic for age, body weight, range from incidence to arriving at hospital, systolic blood pressure, diastolic blood pressure, blood glucose, GCS, SpO2, core temperature, and the length of surgery which were analyzed using one-way Anova test. The sex variable was measured using Chi Square test, and the result was considered significant if $p<0.05$ and very significant if $p<0.001$.

Table 2 shows statistical analysis of glutamate level using paired samples t-test and one-way Anova test. Glutamate level post operative was not different among all groups ($p=0.926$). Whereas, if we see the decrease of glutamate level from pre operative to post operative, in COX2-II group, it showed significant decrease compared to other groups ($p=0.035$).

Figure 1 shows that significant glutamate decrease was in COX2-III group ($\Delta=-2.970$), followed by COX2-II group ($\Delta=-2.918$), COX2-IV group ($\Delta=-1.175$), and COX2-I group ($\Delta=-0.987$), respectively. Increased glutamate level occurred in control group ($\Delta=0.008$).

Discussion

Central inflammation after head injury activates astrocyte and microglia proliferation at the proximal injured area, which then exacerbate tissue damage. Cytokine influences inflammatory cell infiltration and proliferation. Inflammatory cytokine such as IL-1 and IL-6 trigger inflammation right after head injury, while anti inflammatory IL-10 reduces this activity. IL-1β formulate the development of COX-2 at the endothelial, inflammatory cells, and brain epithelial cells. COX-2 may induce proliferation of inflammatory cells and may infiltrate into the central nervous system after trauma, but the role and mechanism of eicosanoid in this

process remains unclear. Consequently, the later activated COX-2 may contribute to infiltrating macrophage and leucocyte, which may implicate on the secondary trauma process and develop further edema, cavities and scar tissues in the long term right after the trauma. (15)

The prostaglandin E receptor subtype EP3 (EP3 receptor) is considered prostaglandin E2 (PGE2) receptor predominantly located in the neuron and scaterred around massively in mouse brain and pig. Out of 4 prostaglandin E receptor subtypes, the EP3 receptor is the strongest bound to the PGE2. EP3 receptor is just next to the responding neuron to IL-1β hipothalamus preoptic anterior mouse brain, supporting EP3 receptor, which may be involved in inflammatory response due to cytokine at the central nervous system (CNS). (15)

Research showed that COX-2 inhibition will decrease COX-2 expression at cortex and hipocampus mouse brain right 72 hours post trauma. This therapy also declined IL-1β level, a cytokine proinflammatory, at the injured brain area 12 hours post trauma. IL-1β is activated by caspase-1 and therefore it is no doubt that there would be bifasic figure following type 3 adenylyl cyclase (AC3). Micro injecting IL-1β at the mouse brain increased inflammatory cells, neuron cell deaths, and vasogenic edema. Histochemistry analysis showed decrease on the expression of vascular endhotelial molecular adhesion, which is exactly the same as what is happening with heart vascular endothelial cells. (15)

Cellular adhesion molecules (intercellular adhesion molecules [ICAM], vascular cell adhesion molecule 1 [VCAM1], and E-selectin) facilitate peripheral inflammatory cells adhesion to the cerebrovascular endothelia, and considered as the first phase of extra passage into the brain. Peripheral infiltrating and proliferating cells, such as neutrophil and macrophage, exacerbate brain injury. In animal lab (pig) with ischemic brain injury, leucocyte decline will decrease mortality rate, repair behavioral improvement and neuropathologic score at day-7 after the trauma. In addition, vasogenic edema is the result of changes in blood brain barrier, which is originated from the interaction of astrocytes pedicles and cerebral vascular endothelia. If endothelial cell and astrocyte metabolism processes were stabilized using P-450 eicosanoids, then the capability in witholding injury and improving blood brain barrier would be maintained. (15)

Activating and/or inhibiting nuclear factor kappa B (NF-κB) transcript factor are considered the inhibiting mechanism of COX-2 for neuron protection. Activating NF-κB would increase transcription from COX-2 level in the neuron tissue. This inhibitor, κB, may be inactivated through phosphorylation or direct oxydation with free radicals. By decreasing prostaglandin products and reactive oxygen species (ROS), COX-2 inhibition might decrease NF-κB activation and further influence its transcription and related apoptosis gene. Additionally, activation of other eicosanoids might involve NF-κB stabilization or inhibit the inhibitor of κB (IκB). So, increase production of brain eicosanoids and COX-2 deficiency reduce peripheral inflammatory infiltration glia proliferation and the development of scar tissue. (15) Adding COX-2 inhibitor in the standard therapy based on Brain Trauma Foundation Guidelines 2007 will reduce edema incidence due to inflammation.

The glutamate level

Glutamate is a primary neurotransmitter in the brain. Glutamate and aspartate are excitatory amino acids, which have the highest concentrate in the extracellular and produced right after traumatic brain injury. There are two mechanisms which produce excitatory amino acids and resulting in cell death or excitotoxicity, including (1) chloride and natrium influx during acute neuron trauma resulting in glial cell edema, (2) influx of calcium ion resulting in delayed neuron cell damage. (7,8)

Glutamate was released from the neuron and will infiltrate into the extracellular compartment when the sodium and potassium gradient extracellular are facing disturbances. High level of glutamate will cause neuron cell depolarization by activating AMPA and NMDA receptors, increasing sodium and potassium ion conduction. NMDA receptors will also cause calcium influx and will trigger further channel damage. Glutamate activates metabotrophic receptors, where through second messenger system may increase the release of calcium from the intracellular and activate other biochemistry processes. The damage is due to high glutamate level. Damage due to high glutamate level is termed excitotoxicity and is resulted from the activation of glutamate receptors and involving ions as well as biochemistry changes. (7)

In addition to increased influx through membrane channel, citophylic calcium level is increased by reducing calcium cell pump and increase release of calcium from intracellular organs, such as mitochondria, and endoplasmic reticulum. It is estimated that high level of citoplasmic calcium will trigger some events, which will further lead to ischemia damage. (7,16,17)

In brain injury, due to decreasing oxygen supply as

opposed to consumption, glutamate level increases. This caused by brain tissue ischemia, disturbance in Na-K pump, membrane depolarization, unavailability of ATP, and over expression of COX-2. (7,11)

Factors causing secondary brain injury like hypertension, hypotension, hyperthermia, hypoxia, and hyperglicemia, did not occur to all research samples, but the over expression of COX-2 still existed. (3) The use of COX-2 inhibitor in this study may reduce glutamate level significantly after giving COX-2 inhibitor in 2 dosages.

Right after brain injury, glutamate is released and may activate inflammation and brain edema, which may lead to death through various pathways. One of the pathways leading to cell death is activation of arachidonic acid, which further triggers leucotriene to attract microglia to release cytokine, such as IL-1, IL-6 and TNF-α. Other pathway will be conducted by prostaglandin and thromboxane, which considered as inflammatory mediator. (7)

Cyclooxygenase-2 (COX-2) is considered as a crucial mediator in glutamate releasing pathway. Glutamate release depends on kalium and involves N-methyl-D-aspartate receptor, which is also one of glutamate receptor. This way, the COX-2 inhibitor protects the neurons directly by decreasing glutamate as cellular response. (18)

Controlled factors

Controlled factors were blood pressure, oxygen saturation, hypercarbia, hypoxemia, and body temperature.

1. Blood pressure

Systolic and diastolic blood pressure did not show significant differences in COX2-1, COX2-2, COX2-3 and COX2-4 groups, which further clarified no secondary brain injury effects due to hypotension or hypertension. (6,19)

Hypertension may increase brain blood flow, brain blood volume, increase brain edema and intracranial pressure. Therefore, if hypertension exists, think of hypertension as part of Cushing's triad (decrease awareness, bradycardia, and hypertension). Try reducing intracranial pressure first, and if the hypertension is not due to increased intracranial pressure, then manage and treat hypertension since hypertension is considered as risk factor in the development of brain edema and intracranial pressure. Blood pressure in this case was reflected by cerebral perfusion pressure (CPP), which must be maintained between 60-70 mmHg. If CPP is higher than 70 mmHg, it may precipitate acute respiratory distress syndrome (ARDS) and trigger inflammation. (5,19) There were no hypertension in these all study groups, therefore there were no influence on IL-1β or glutamate levels due to changes in blood pressure.

Blood pressure reduction will cause significant decline in oxygen supply to the brain, since CPP=MAP-ICP. Consequently hypotension will trigger increased intracranial pressure reflex due to brain ischemia and cerebral infark. (6,19) Hypotension reduces blood flow to the brain and it is mentioned in the Brain Trauma Foundation Guidelines 2007 that cerebral perfusion pressure must not less than 50 mmHg and/or systolic blood pressure must not less than 90 mmHg. Systolic blood pressure less than 90 mmHg or CPP less than 50 mmHg will precipitate cerebral ischemia. (6,19) In Brain Trauma Foundation Guidelines 2016 maintaining systolic blood pressure at ≥100 mmHg for patient 50- to 69-year-old or at ≥110 mmHg or above for patient 15- to 49- or >70-year-old may be considered to decrease mortality and improve outcome. Recommended target CPP 60-70 mmHg. (20)

In these 5 study groups, there were no significant variable differences and no decline in systolic blood pressure less than 110 mmHg, which meant that there was no secondary brain injury due to hypotension. Also there were no hypertension, which might induce brain edema and increased intracranial pressure that might lead to neuron cell death, or hypotension, which might induce increased intracranial pressure reflex and brain ischemia, that might lead to neuron cell death. Through those 2 different mechanisms, brain tissue damage will occur and there will be inflammation process and increased COX-2 level in brain tissue. Therefore, there would be no significant differences in blood pressure figures, and consequently since blood pressure figures across all study groups were considered similar, there were no influence from blood pressure to the glutamate levels.

2. Core temperature

Patients with intracranial hypertension risk factor, like patients with brain injury, are influenced by changes in body temperature due to increased blood flow to the brain along with increased body temperature. Increased blood volume to the brain related to increased body temperature may increase intracranial pressure and may cause secondary injury. Since hyperthermia will increase neuron cell damage risk, patients may impose risk of facing secondary brain injury through increased intracranial pressure. Decrease in body temperature will slow down cerebral metabolic rate, which means

lowering blood flow to the brain. Every decreasing of 1 ºC, brain blood flow will decline around 5%. (20,21)

Post ischemic hyperthermia is closely related to the increased size of the infarct, and worst outcomes. Even though tight control of normal body temperature has been noted as crucial therapeutic strategy referring to the Guidelines for the Management of Severe Head Injury, clinical management strategy is frequently ineffective and may be considered contraindicated for patients with brain injury. Management to be normothermia (fever healing using intravascular cooling) is considered effective in heat reduction strategy and severity of secondary brain injury after severe brain trauma due to increased intracranial pressure and fever. (22,23)

It is well known from several experimental studies that hypothermia has a neuroprotective effect after brain ischemia, although the detailed mechanism remains unclear. Hypothesis is made due to decrease in brain metabolism, avoid cell apoptosis, decrease mytochondria dysfunction and free radical production as well as DNA oxidative damage, decrease calcium influx, decrease release of glutamate exitatory amino acids (EAA), avoid lipid peroxidation, and decrease edema development. Commonly, neuroprotective hypothermia effect in global and focal ischemia is received after brain injury. Hypothermia is also believed to be utilized in controlling increased intracranial pressure and block biochemistry cascade during secondary brain injury process. (24)

Brain protection mechanism through hypothermia is possibly by slowing down brain metabolism and anoxia/ischemia depolarization, maintain ion homeostasis, lowering excitatory neurotransmission, prevent or decrease secondary damage due to biochemistry changes. Hypothermia parameter which is believed has brain protection effect is 35.5-36 ºC. (21) In all study groups, temperatures were measured around 35.5-36.6 ºC starting from pre surgery to day-3 post surgery, and therefore there would be no bad impact from hyperthermia that increased inflammatory effect in the brain.

3. Random blood sugar test

Hyperglicemia is frequently occurred in brain injury. Increase in blood sugar level may occur as the body response towards increased circulated catecholamine and cortisol level, which protrude after brain injury. Insulin release is decreased by high level of catecholamine and steroid, which further induce gluconeogenesis process. Hyperglicemia may reflect the size and severity of brain injury. The more severe brain injury, the higher level of catecholamine is released. There is feedback mechanism between GCS score with serum catecholamine level. (6,19)

Brain injury patients with GCS ≤8 and blood sugar level >200 mg% are related to worst outcome. Providing glucose containing solution may exacerbate severe ischemic brain damage in animal laboratory. Patients with persistent hyperglicemia have worst outcome compared to patients with normal blood sugar. Hyperglicemia also correlates with outcome after brain injury: the higher blood sugar, the worse the outcome. Therefore, maintain the blood sugar level not more than 150 mg%. Provide glucose only when hypoglicemia exists (blood sugar <60 mg%). (6,19)

Glucose is the main energy source, either produced by aerobic or anaerobic process. Lots of researches show that increasing blood sugar before the ischemia insult will only worsen neurologic outcome. Even though the mechanism is unclear, there are some underlying theories saying that with the ischemia, glucose will be metabolized anaerobically, and will produce lactate accumulation. Increased lactate level will reduce intracellular pH, disturb cellular functions, and finally lead to cell death. However, later studies failed to correlate lactate level in the serum with the severity of neurologic outcomes. Alternatively, general decline in blood flow may be conducted. Other studies mentioned that hyperglicemia reduced cerebral adenosine level. Adenosine is an inhibitor of EAA release, which play crucial role in ischemic cell death. So, providing glucose in brain surgery patients is considered appropriate only when indicated, for instance when blood sugar level is <60 mg%. Commonly, blood sugar is maintained not higher than 150 mg% (100-150 mg%). Intraoperative hypoglicemia will not occur in less than 4-hour surgery and normal blood sugar patients. Hyperglicemia has bad effect on body homeostasis, therefore if blood sugar is >200 mg%, provide insulin. (6,19)

In this study, each of COX2-II, COX2-IV and control groups had significant value of variables in random blood sugar level, despite of various blood sugar levels from 94-136 mg% in post surgery day-1 to day-3. Across all study groups, random blood sugar levels revealed around 146-181 mg%, and therefore, even with significant various level of random blood sugar, clinically it would not lead to secondary brain injury, which in turn would influence glutamate level.

Conclusion

Selective COX-2 inhibitor given intravenously had brain protection effect in patients with moderate traumatic brain injury based on the capability of COX-2 inhibitor in reducing glutamate level.

Table 1. General characteristic

General characteristic	Groups					p-values
	Control n=6	COX2-I n=6	COX2-II n=6	COX2-III n=6	COX2-IV n=6	
Age (year), average (SD)	36.17 (16.68)	31.67 (13.62)	24.67 (12.61)	28.33 (16.21)	26.83 (9.37)	0.650
Sex, n (%) - Male - Female	 5 (83.30) 1 (16.70)	 5 (83.30) 1 (16.70)	 5 (83.30) 1 (16.70)	 6 (100) 0 (0)	 5 (83.30) 1 (16.70)	0.886
Body weight (kg), average (SD)	65.00 (10.49)	62.17 (8.50)	58.67 (7.12)	61.67 (9.31)	64.17 (11.14)	0.798
Incidence range (h), average (SD)	9.00 (2.61)	11.00 (2.53)	12.00 (5.18)	10.17 (4.71)	8.00 (3.52)	0.389
SBP (mmHg), average (SD)	118.67 (12.24)	137.33 (32.63)	117.17 (30.33)	120.67 (22.99)	122.67 (16.48)	0.617
DBP (mmHg), average (SD)	75.17 (6.15)	72.5 (10.62)	63.33 (20.1)	72.83 (11.43)	78.50 (5.79)	0.287
Blood glucose (mg%), average (SD)	181.33 (40.27)	142.5 (8.62)	139.67 (33.1)	138.33 (28.39)	160.5 (26.4)	0.079
GCS, median (SD)	11.00 (1.26)	11.17 (1.17)	10.50 (0.84)	10.50 (1.22)	11.50 (1.38)	0.535
Core temp (^{o}C), average (SD)	35.62 (0.84)	35.98 (1.01)	36.30 (0.53)	36.35 (0.67)	36.53 (0.79)	0.308
SpO2 (%), average (SD)	100 (0)	100 (0)	100 (0)	99.67 (0.82)	99.83 (0.41)	0.537
LOS (h), average (SD)	2.61 (0.45)	2.58 (0.49)	2.56 (0.35)	2.63 (0.43)	2.57 (0.34)	0.998

Legend: p-values were obtained from one-way Anova test, except sex variable using Chi Square. Significant difference was reached if $p<0.05$ and very significant if $p<0.01$. SD=standard deviation; Control=NaCl 0.9% was given; COX2-I=COX-2 inhibitor was given once/day; COX2-II=COX-2 inhibitor was given twice/day; COX2-III=COX-2 inhibitor was given thrice/day; COX2-IV=COX-2 inhibitor was given 4 times/day; SBP=systolic blood pressure; DBP=diastolic blood pressure; GCS=Glasgow coma scale; LOS=length of surgery.

Table 2. Pre and postoperative glutamate levels

Groups		Glutamate levels		p-values
		Preoperative	Postoperative	
Control	Mean (SD)	7.33 (4.504)	7.34 (4.116)	0.809[a]
	Median (range)	6 (3-13.7)	6.8 (3.45-12)	
COX2-I	Mean (SD)	6.71 (3.91)	5.73 (1.821)	0.577[a]
	Median (range)	5.09 (3.14-13.66)	5.48 (3.7-8.9)	
COX2-II	Mean (SD)	8.13 (3.268)	5.21 (3.662)	0.035[a*]
	Median (range)	9.08 (3.22-12.3)	4.42 (1.88-11.1)	
COX2-III	Mean (SD)	11.47 (9.509)	8.50 (6.956)	0.576[a]
	Median (range)	9.59 (0.5-27.45)	7.38 (1.88-19.7)	
COX2-IV	Mean (SD)	10.74 (8.49)	9.57 (5.98)	0.620[a]
	Median (range)	8.96 (3.41-26.8)	8.85 (3.52-19.7)	
p-values		0.258[b]	0.926[b]	

Legend: [a]=p-values were obtained from paired samples t-test; [b]=p-values were obtained from one-way Anova; [*]=significant difference ($p<0.05$); Control=NaCl 0.9% was given; COX2-I=COX-2 inhibitor was given once/day; COX2-II=COX-2 inhibitor was given twice/day; COX2-III=COX-2 inhibitor was given thrice/day; COX2-IV=COX-2 inhibitor was given 4 times/day.

Figure 1. Glutamate level changes (pre and post) in all groups

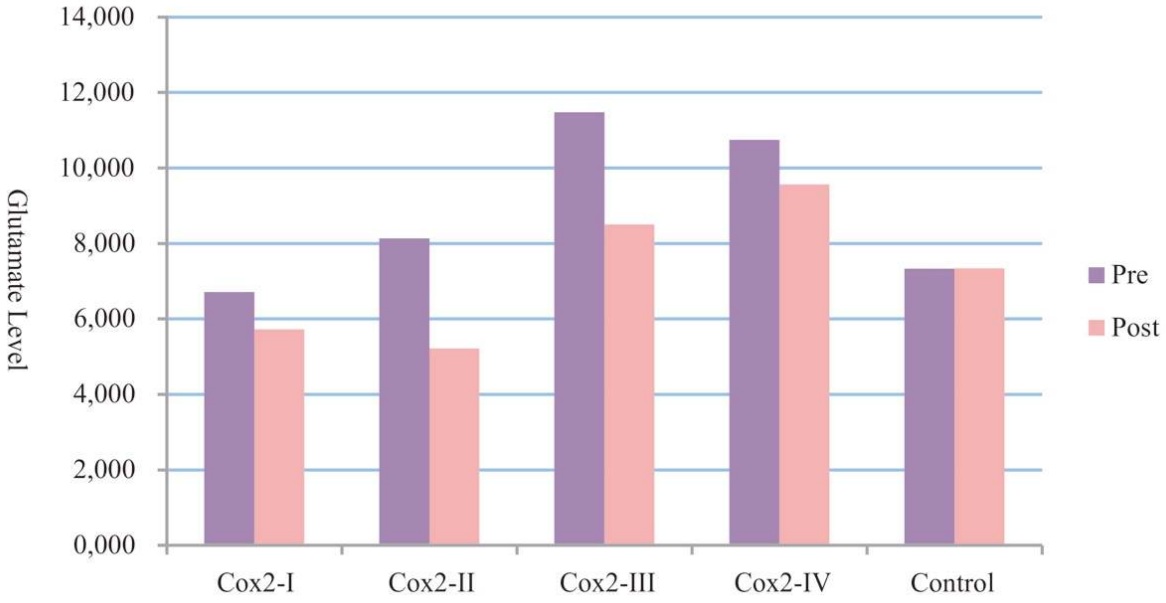
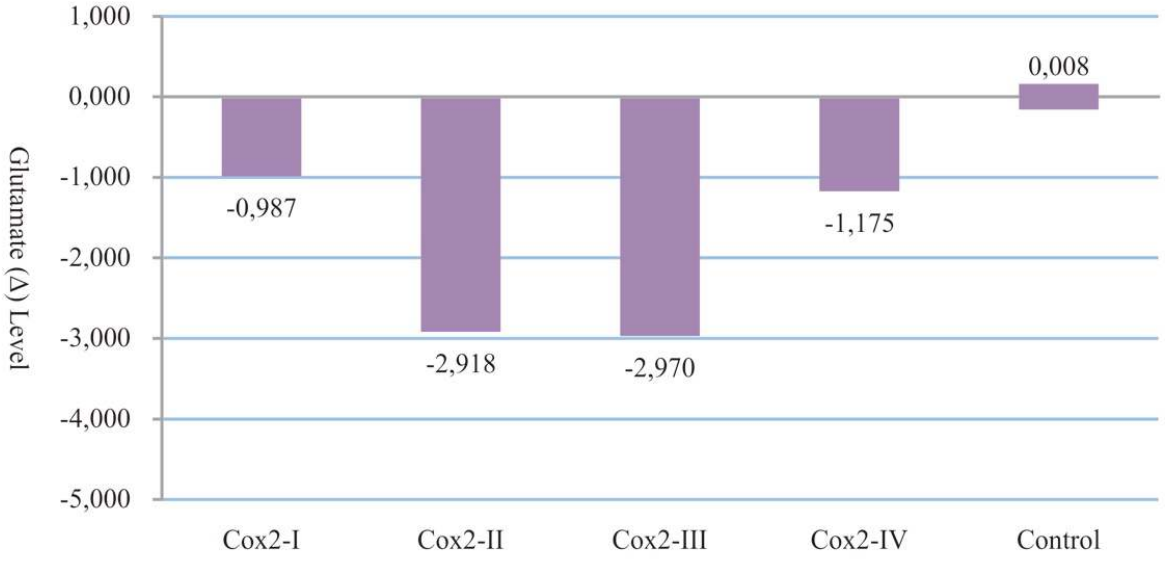


Figure 2. Comparison of glutamate changes (pre and post) in all groups



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