ORIGINAL ARTICLE

The Role of Dexmedetomidine as Brain Protector Assessed by Cortisol, IL-6 and COX-2 Concentration in Rat Model of Traumatic Brain Injury

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ABSTRACT

Background: Many studies over the world perform to find the best drugs for brain protection, but so far the result still inconsistent. The objective of this study is to evaluate the role of dexmedetomidine as brain protector based on cortisol, IL-6 and COX-2 plasma concentration.

Methods: A laboratory experimental study with randomized control trial design had been conducted on 24 male, 3 month wistar rat, receive standard artificial brain trauma. Wistar rat divided into three group, K1 (NaCl), P (treatment) and K2 (control). Rat anesthetized with ketamin 80 mg/lgBW intra peritoneally, followed by artificial brain trauma for group K1 and P, after skin stitch group K1 receive saline intra peritoneal, and group P get dexmedetomidine 60 µgr/kgBW. K2 group as control group were not received any trauma nor medication. Dexmedetomidine or saline was given serially at 3 hours, 12 hours and 24 hours after trauma. Blood for ELISA analysis taken at pre-trauma, 12 hours and 24 hours after trauma.

Result: Cortisol were unsignifficantly increased in K1, 12 hours after trauma compared to P group. IL-6 concentration in K1 increase significantly 12 and 24 hours after trauma. (160±15,57) vs (140,5±17,65) and (172,6±19,07) vs (124,2±23,6). Cox-2 concentration in K1 increase significantly 12 and 24 hours after trauma. (1491,41±341) vs (803,62±215,73) and (1048,45±170,43) vs (588,93±198,57.). Spearman's analisys showed positive correlation between IL-6 and cortisol (ρ=0,83), IL-6 and COX-2 (ρ=0,71), COX-2 and cortisol (ρ=0,71) 12 hour after trauma.

Conclusion: Dexmedetomidine has brain protection effect through decreasing of IL-6, cortisol and COX-2 concentration.

Keywords: brain protection, dexmedetomidine, IL-6, COX-2

INTRODUCTION

After a head injury, there will be many patophysiologic response, one of them is cytokine release, a main mediator release at the beginning inflamation process due to trauma. Some cytokine had been researched in related to head injury, a proinflammation cytokin which are, for example, *interleukin-1* (IL-1), IL-6, IL-8, and *tumor necrosis factor-α* (TNF-α).[1,2]

Interleukin-6 and TNF- *α*were released after head injury, and if the patients with the GCS less than 8, showed IL-6 lever higher. The cytokine released after traumatic head injury will stimulate arachydonic acid with the help of COX-2 will be altered to prostaglandin which showed inflammation process, which lead to secondary trauma[3,4.]

In head injury, there also norepinephrine released which escalates pro inflammation cytokines like IL-1, IL-6, and TNF-α, also Hypothalamic Pituitary Adrenal Axis activation, marked by increasing cortisol level, while inflammation processed marked by the increase of IL-6 and COX-2. That means there is relation between neuroendocrine, immune system, and inflammation that worsen brain injury [5,6]

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Dexmedetomidine is second generation α2 agonis selective for α2 adrenergic receptor (1600 : 1 for α2 : α1). Primary effect of stimulating α-2 agonis receptoris slowing norepinephrin release which made less excitation in central nervous system. Dexmedetomidin can suppress central and also peripheral sympathic tone. The research using lab rat during ischemic dexmedetomidin will supressnorepinephrin up to 95 % compare to control[3,7,8]

Dexmedetomidin has brain protection effect proven in several research, because it reduce Norepinephrin release, which had connection to its ability to protect the brain.[9,10]

Dexmedetomidin role in neuroprotection in brain injury observed by investigating its effect in reducing post trauma sympathetic response, not to its inflammation effect. This research aimed to assess dexmedetomidin role as brain protector by evaluating cortisol level, IL-6 and COX-2 in rat model with traumatic brain injury.

MATERIAL AND METHODS

Subject of this research are 24 male 3 moths, old wistar rats with average weight 250 – 350 gram. The rats came from *Laboratorium Penelitiandan Pengujian Terpadu (LPPT)* unit IV, UGM, Yogyakarta.

After written consent from medical ethics research board, we divided the rats into 3 groups, each consist of 8 rats. K1 group = positif control group which the rats had 0,9% NaCl post trauma. P group which the rats had dexmedetomidin60 µgr/kg post trauma. K2 groups = negative control group, which don't have any medication nor trauma. For 2 weeks, all rats were adapted, then for the K1 and P groups, performed craniectomy then given 2,5 gr weight to its duramater which cause brain injury. Each group then had the medication as their protocol

intraperitoneal after wound suture, 3 hours – 12 hours and 24 hours post trauma. Vital signs (pulse, respiration rate, O2 saturation) were observed prior to trauma, after wound suture, and then 3 hours – 12 hours and 24 hours post trauma. Cortisol level, IL-6 and COX-2 were observed before injury, 12 hours and 24 hours after brain injury through orbita vein using *Mouse IL-6 ELISA BMS 603, Bender Med System, (Burlingame,USA)* and also Cusabio Biotech for examining cortisol level and COX-2.

RESULTS

Table 1 is Demographic datas consisted of weight, length of measure to make acquired brain trauma, respiration rate, temperature, and saturation for all groups, showed no significant differences (p> 0,05).

Table 2 and table 3 showed rate and standard deviation for pulse (minutes) and respiration rate (minutes) during observation. The result counted using Anova and Friedman showed No. significant differences (p > 0,05) foreachgroups.

Tabel 4 showed mean value and standard deviation of temperature during observation. Friedman Analysis showed significant differences between P group (dexmedetomidin) (p = 0,018) before trauma and 3 hours post trauma.

Table 5 presented the mean values and standard deviations of oxygen saturation among the three groups during the observation. The results of the statistical test ANOVA and Friedman showed significant difference (p <0.05) in the observed variables.

From the statistical analysis by ANOVA appears that there is no significant difference in cortisol levels between the three groups prior to treatment. There is a significant difference (p <0.05) cortisol levels between the three groups at 12 hours post-trauma. When analyzed by the Post Hoc, seem significant differences occurred between the groups K1 and K2, and group P and K2. (Table 6 and Figure 1).

There was a significant difference (p <0.05), serum IL-6 in the third group at 12 hours and 24 hours after trauma. From the pictures. 2 it appears that IL-6 at higher NaCl clusters of clusters P (Dex) and a control group. Observations for each group indicates that the group K1 (NaCl) no differences in the mean serum IL-6 before trauma with 12 hours and 24 hours after trauma. Whereas in group P (Dex) and K2 group (control) no significant difference was found (p> 0.05) between pre-trauma with 12 hours and 24 hours post-trauma.

Differences in the mean serum IL-6 at 12 hours and 24 hours if followed by post hoc analysis of data obtained significant difference occurred between the groups with group P K1, K1 and K2 groups

There was a significant difference (p <0.05) the rate of COX-2 between the three groups at 12 hours after trauma and 24 hours after trauma. Post Hoc analysis appears that a significant difference in 12 hours occurred between K1 clusters with cluster P and between both clusters with cluster K1 K2. From the pictures. 3 it appears that the rate of COX-2 in the group of NaCl higher than group P (Dex) and a control group. Observation for each group indicates that the group K1 (NaCl) has no differences in the mean change rate of COX-2 by trauma with 12 hours and 24 hours after trauma. Whereas in group P (Dex) and K2 group (control) have no significant difference (p> 0.05) between pre-trauma with 12 hours and 24 hours post-trauma.

From variables : IL-6, cortisol and COX-2, correlation test was done to see the relationship between two variables and the correlation between them. Correlations were analyzed based on the changes that occur after exposure to trauma in each group. Changes that occured after 12 hours in the K1 group showed no correlation by Spearman's analysis. Datas are presented in the following figures for the relationship between variables NaCl group at 12 hours post-trauma. Data on the group K1 (NaCl) if further elaborated as shown in the graph below(Picture 4). The graph and the regression equation appear that the correlation between these variables are all positive correlation.

After the analysis of correlation in group P (dex) and K2 group (Control), both groups showed no correlation between the variables of cortisol, IL-6 and COX-2

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Table 1. Demographic Data Observed Before Treatment

Variables	**K1 (NaCl)**		**P (Dex)**		**K2 (Control)**		***P value*** **(Anova)**
	Mean Value	**SD**	**Mean Value**	**SD**	**Mean Value**	**SD**	
Weight (gram)	282,38	12,070	282,63	13,384	280,75	10,754	0,945
Length of measure (minutes)	18,25	1,488	18,38	1,188	18,13	1,727	0,945
Pulse	179,38	4,406	178,88	2,949	179,0	2,390	0,953
Respiration rate	59,75	1,982	59,75	1,982	59,75	1,982	1,000
Temperature	36,96	0,074	36,95	0,141	36,96	0,130	0,971
Saturation	97,38	0,518	97,38	0,518	97,38	0,518	1,000

SD= Standard Deviation

Table 2: MeanValueand Standard DeviationPulse per mins during Observation

Observation	**ResearchSubject**						**P value (Anova)**
	K1 (NaCl)		**P (Dex)**		**K2 (control)**		
	Mean Value	**SD**	**Mean Value**	**SD**	**Mean Value**	**SD**	
Pre trauma	179,38	4,406	178,88	2,949	179,00	2,390	**0,953**
Post trauma	179,50	3,742	178,63	1,768	178,88	1,727	**0,787**
3 hours post	176,75	6,756	177,75	2,172	178,63	1,768	**0,691**
12 hours post	179,25	2,375	176,75	2,605	179,75	2,493	**0,056**
24 hours post	182,25	4,334	178,50	2,330	181,00	2,138	**0,069**
p value (Friedman)	**0,697**		**0,252**		**0,179**		--

SD= standard deviation

Table 3: Rate and Standard DeviationRespirationrateeachminsduringobservation

Observation	Research Subject						p-value (Anova)
	K1 (NaCl)		P (Dex)		K2 (control)		
	Mean Value	SD	Mean Value	SD	Mean Value	SD	
Pre trauma	59,75	1,982	59,75	1,982	59,75	1,982	1,000
Post trauma	59,50	2,070	59,50	2,070	59,75	1,982	0,961
3 hours post	59,75	2,915	59,25	4,132	60,50	2,330	0,739
12 hours post	60,25	2,493	60,00	2,619	61,00	2,390	0,711
24 hours post	62,75	3,196	60,25	2,493	62,00	4,408	0,351
p – value (Friedman)	0,169		0,890		0,518		--

SD= Standard Deviation

Table. 4 Mean value and Standard Deviation of Temperature during observation

Observation	Research Subject						p- value (Anova)
	K1 (NaCl)		P(Dex)		K2(control)		
	Mean Value	SD	Mean value	SD	Mean value	SD	
Pre trauma	36,96	0,074	36,95	0,141	36,96	0,130	0,971
Post trauma	37,01	0,083	36,90	0,107	37,11	0,107	0,053
3 hours post	36,90	0,107	36,76	0,119	37,11	0,69	0,258
12 hours post	36,95	0,141	36,85	0,169	37,01	0,125	0,105
24 hours post	36,96	0,177	36,85	0,093	37,02	0,19	0,108
p- value (Friedman)	**0,364**		**0,018**		**0,958**		--

SD = standard deviation

Table 5: Mean Values and Standard Deviation of Oxygen Saturation during observation

Observation	Research Subject						p-Value (Anova)
	K1 (NaCl)		P (Dex)		K2 (Control)		
	Mean value	SD	Mean value	SD	Mean value	SD	
Pre trauma	97,38	0,518	97,38	0,518	97,38	0,518	1,000
Post trauma	97,13	0,641	97,25	0,463	97,13	0,641	0,887
3 Hours post	97,38	0,518	97,50	0,535	97,50	0,535	0,863
12 Hours post	97,13	0,354	97,38	0,518	97,50	0,535	0,296
24 Hours post	97,50	0,535	97,38	0,518	97,38	0,518	0,860
p-Value (Friedman)	**0,556**		**0,910**		**0,582**		--

SD= Standard Deviation

Table 6: Mean Values and Standard Deviation of Cortisol Level

Observation Time	Kortisol (ng/ml)						P Value Anova	Post Hoc
	K1 (NaCl)		P (Dex)		K2 (Control)			
	Mean Value	SD	Mean Value	SD	Mean Value	SD		
Pretrauma	23,155	1,35	22,853	2,84	23,576	0,88	0,747	K1.K2
12 hours	21,373	4,02	18,51	3,43	25,615	1,97	0,001	P-K2
24 hours	17,868	3,12	16,045	5,48	18,636	5,26	0,543	
*P*value Friedman	0,034		0,030		0,021			

D= Standard Deviation, 12 hours Post HocAnalysis: K1-K2 (p=0,019) ; P-K2 (p=0,00)
Wilcoxonanalysis K1= 24 hourspre trauma, 12 hours-12hours, P= 12hourspretraumadan 24hours, K2= 12 hourspre, 24 hourspreand 12 hours – 24 hours.

Figure1: Mean Value Graph of Cortisol Level During Observation

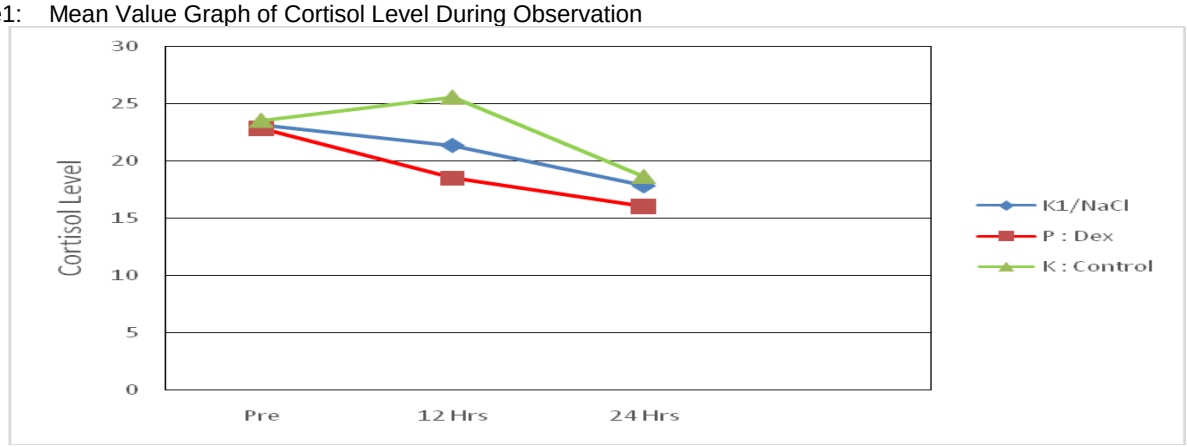

Table. 7 Mean value and Standard Deviation IL-6 Level

Observation Time	IL-6 (pg/ml)						P Value (Anova)	Post Hoc
	K1 (NaCl)		P (Dex)		K2(Control)			
	Mean Value	SD	Mean Value	SD	Mean Value	SD		
Pre trauma	133	24,071	143	16,937	137,5	29,135	**0,708**	
12 hours	160	15,575	140,5	17,655	129,5	21,078	**0,010**	**K1.P;K1-K2**
24 hours	172,6	19,071	124,2	23,602	130,5	24,372	**0,001**	K1.P;K1-K2
P Value Friedman	**0,000**		**0,206**		**0,908**		--	

SD=Standard deviation, 12 Hours Post Hoc analysis : K1-P (p=0,044), K1-K2 (p=0,003). 24 hours Post Hoc analysis : K1-P (p=0,000), K1-K2(p=0,001)Wilcoxon Analysis: K1= pre-12 hours and 12 hours-24 hours.

Table 8: Mean Value and Standard Deviation of COX-2 Level

Observation	COX-2 (pg/ml)						P Value Anova	Post Hoc
	K1 (NaCl)		P (Dex)		K2 (Control)			
	Mean value	SD	Mean value	SD	Mean value	Sd		
Pra trauma	847,54	370,53	849,03	614,1	748,67	198,57	0,867	
12 hours	1491,41	341,33	803,62	215,73	563,18	319,43	0,000	K1.P;K1.K2
24 hours	1048,45	170,43	588,93	198,57	751,91	305,79	0,003	K1.P;K2.P
p values Friedman	**0,044**		**0,073**		**0,093**			

SD= standard deviation, 12 hours post trauma post hoc analysis K1-P (p=0,000), K1-K2 (p=0,000)
24 hours post trauma Post Hoc analysis K1-P (p=0,000), K1-K2 (p=0,000)Wilcoxon analysis: K1=pre-12 hours and 12 hours-12 hours.

Figure 2: Mean ValuesGraph of IL-6 during Observation

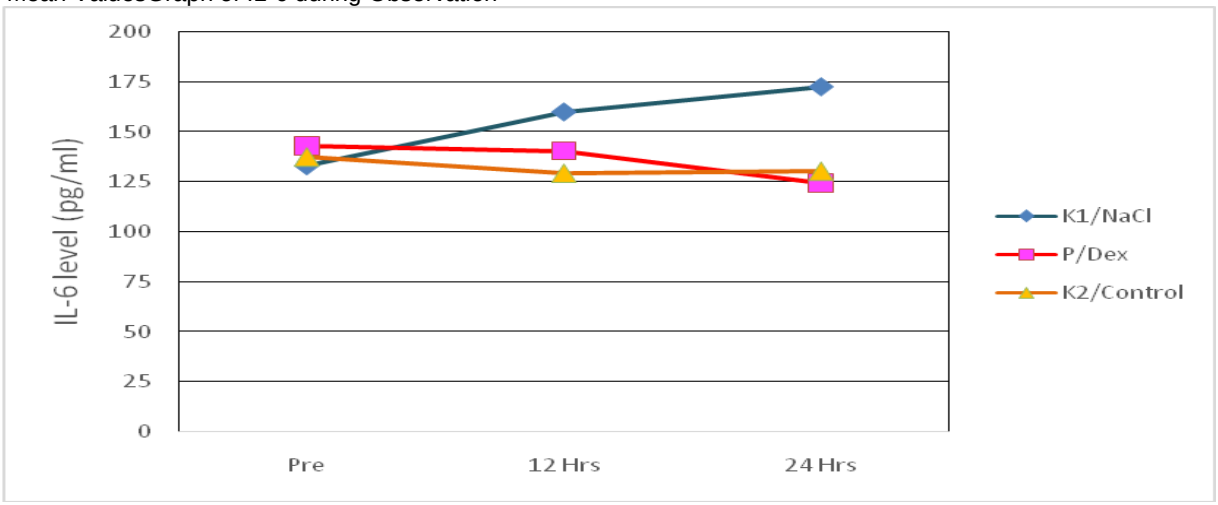

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Figure. 3 Mean Value and Standard Deviation of COX-2 level during observation

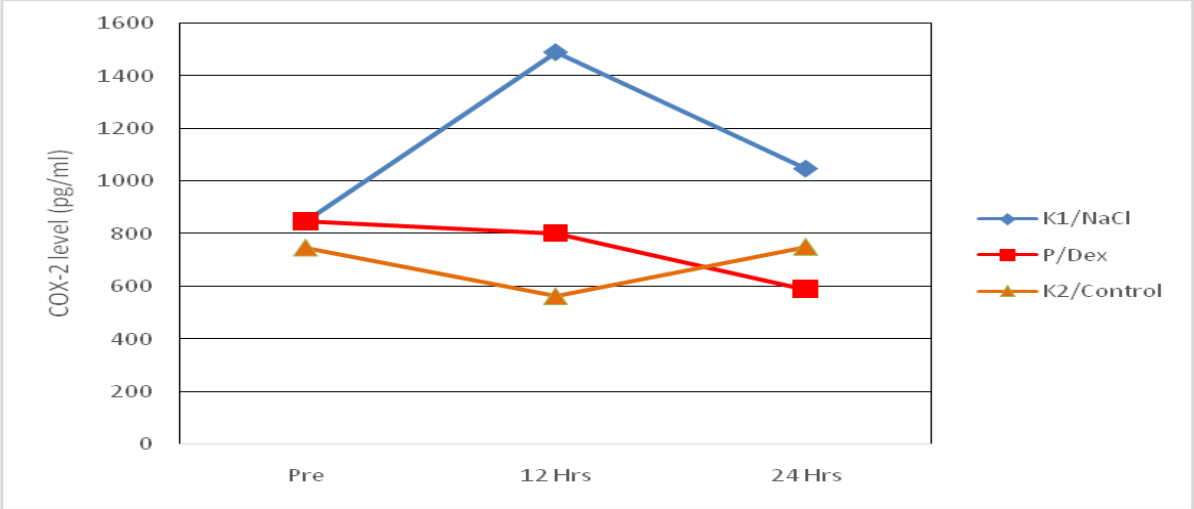


Figure 4 Correlation between IL-6 and COX-2, in K1 group after 12 hours observtion

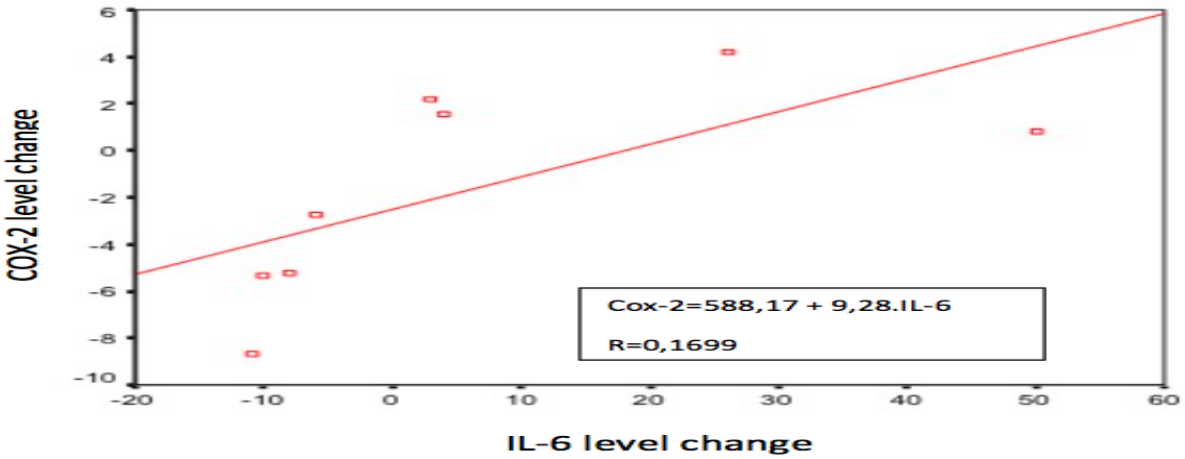


Figure. 5 Correlation between IL-6 and Cortisol in K1 groups after 12 hours post trauma

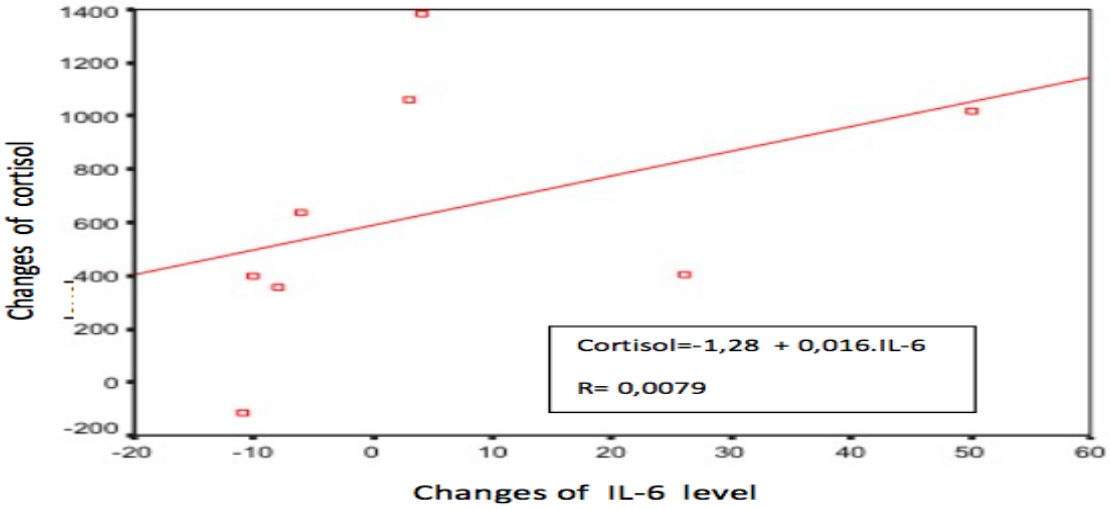
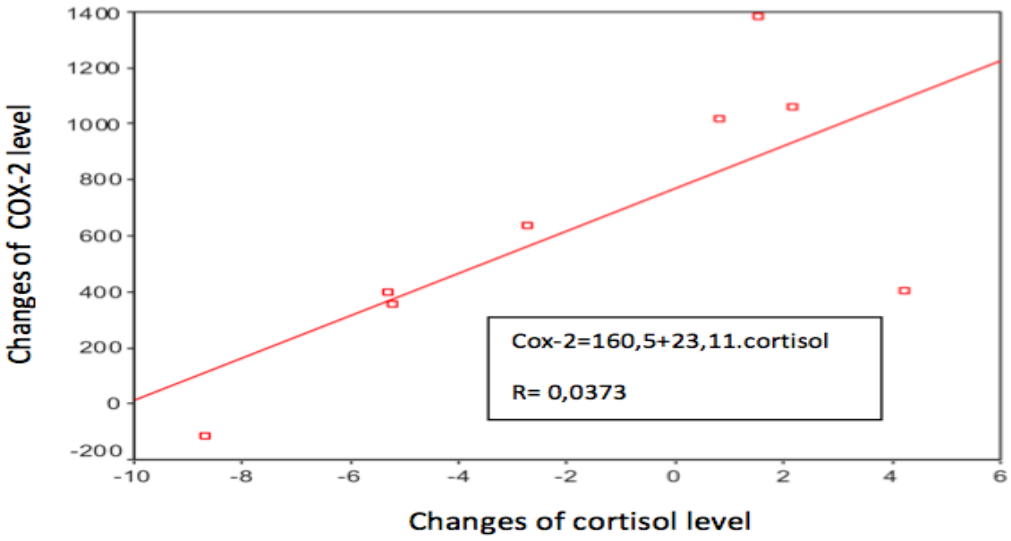


Figure 6 Correlation between Cortisol andCOX2 in K1 groups after 12 hours post trauma.



DISCUSSION

There's decrease of 3 hours temperature after given dexmedetomidin in group P. This happen because dexmedetomidine has sedative and anxiolitic beside slowing metabolism. The increase of post trauma cortisol level showed elevation of ACTH level, although it's only temporary. Many had conducted research on cortisol secretion, which has significant correlation with the degree of trauma, and the glascow coma scale (GCS) on head trauma patients[11,12].

Cortisol also known as stress hormon since it's release when there's stress response. Stress is a term defined when there's hormonal changes and metabolic response after trauma, part of sistemic response which combine endocrine – immune and hematologic response. Stress response is a mechanisme of survival by catalize carbohydrate, lipid, protein and water fluid retention until the body gain full recovery. Although during anesthesia practice is still in doubt the necessity of living creature having this stress response, but in clinical practice, we tried to minimize or make free of stress response to a patient[13].

The result of comparison of cortisol level between the 3 groups showed that in the 24 – hours observation there is No. significant difference, with post hoc analysis known that the significant differences happened only between K1 to K2 group, and K2 to P group. The K2 group which is the control grop of cortisol level, there's elevation in 12 hours observation, aftera significant decrease at 24 hours. This condition can be caused by several things, among others, due to the circadian cycle showed an increase in cortisol levels in the morning and afternoon and then fell in the afternoon or evening, when the study was the morning then observed for up to 24 hours. Besides, the possibility ofexternal factors that can cause stress even though the mice did not get a trauma, such as temperature and light, or environmental enclosure for observation, this condition can increase the production of the hormone cortisol as stres. Mouse is a nocturnal animal, if the possibility of observation during the daytimecanalsoaffectcortisol levels.[14,15,16]

There is a significant difference ($P < 0.05$) levels of IL-6 in all three groups at 12hours and 24 hours after trauma. From Figure 2 it appears that the levels of IL-6 in group NaCl higher than group P (Deks) and the control group. Observations for each group showed that the group K1(NaCl) was no significant difference of IL-6 levels before trauma with 12 hours and 24 hours after trauma. While in group P (Deks) and K2 group (control) did not obtain significant differences ($p>0.05$) between the pre-trauma with 12 hours and 24 hours post-trauma.

Significant differences in levels of IL-6 at 12 hours and 24 hours post-trauma if followed by a post hoc analysis of data obtained significant differences occurred between: groups with group K1 to P, and K1 to K2 group. (Table 7 and Figure 2).

Inflammatory mediators in normal circumstances have low levels, and only slightly affects the healthy tissue but after the injury, will soon be increased proinflammatory cytokines and other mediators with an important role in the inflammatory process in the central nervous system. Proinflammatory cytokines IL-6 greatly affect the expression of cyclooxygenase(COX-2) in the brain and this happened 3 hours after injection ofIL-6[17,18].

Levels of IL-6 to increase after trauma compared with other groups as well as in the NaCl group, indicating a systemic response to trauma through the activity of proinflammatory cytokines.[19]

Dexmedetomidine who has the ability to reduce levels of norepinephrine in the locussereleus workthrough, which will further reduce sympathetic activity, this effect is a mechanism as a protector of the brain, with the mechanism of the formation of proinflammatory cytokines decreases so as to reduce the inflammation that occurs. [6,19]

Cyclooxygenase-2 (COX-2) is the enzyme that produces prostanoid and plays a role in the toxicity as a result of inflammation. Observations levels of COX-2 showed no differences in the three groups ranging from 12 hours post-trauma and increased COX-2 appears to be higher in the saline group compared to the group receiving dexmedetomidine. Although there was a decrease in the NaCl group at 24 hours, but the levels are still significantly higher than the group dexmedetomidine.

Prostaglandins are inflammatory mediators produced through the metabolism of arachidonic acid with the help of enzymes cyclooxygenase and prostaglandin synthesis enzymes. The study examines the effects of inhibition of COX-2 as a protector of the brain have been carried out, using preparations of antiCOX-2, so if dexmedetomidine also can prevent an increase in COX-2 after brain injury, it can objective evidence neuroporotektor effect of dexmedetomidine[17].

There is a positive correlation between cortisol, IL-6 and COX-2 in the group receiving saline at 12 hours after injury. The results of a positive correlation between the three variables (cortisol, IL-6 and COX-2) only in the group K1(NaCl), this was due to a response to the trauma without being affected / inhibited by NaCl, whereas the P group interaction with dexmedetomidine changes which affect the levels of these three variables. Dexmedetomidine an α-2 receptor agonist which is a norepinephrine inhibitor that can reduce stress and inflammatory responses after trauma / injury, this can reduce the increase in IL-6 and COX-2 as well as cortisol that if these three things are correlated then there is a strong correlation results obtained.

CONCLUSIONS AND RECOMMENDATIONS

Dexmedetomidine has effect as protector of the brain through the mechanism of catecholamine activity by lowering cortisol levels and reduce inflammation by lowering levels of IL-6 and COX-2.

Dexmedetomidinecan be used as a protector of the brain in clinical practice, and research is still open to see the long-term effects of dexmedetomidine for more than 24hours.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgement: We appreciate the laboratory technician help ofSugiyonoand statistical help of Darminto.

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