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Adrenocorticotrophin Hormone$_{4-10}$ synthetic inhibit ProstaglandinE$_2$ and IL-1β levels in LPS-induce meningitis

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ABSTRACT: *Bacterial meningitis is still a health problem throughout the world, despite being applied to the newest generation of anti-infective drugs. Intracerebral inflammation is need a lot of attention due to bacterial products released during bacterial lysis could trigger a cascade of inflammatory. Anti-inflammatory drug is a logical reasoning to improve the outcome of disease. ACTH$_{4-10}$ synthetic is identified as neuropeptide, identically with aMSH exogen because α-MSH shares the first 13 amino acids sequence with ACTH$_{4-10}$ and receptor was used to have a series of functions anti-inflammatory effects. Purpose of this study is to determine the effect of administration ACTH$_{4-10}$ synthetic on levels of PGE$_2$ and IL-1b as proinflammatory mediators in LPS-induced meningitis in animals. Research methods, experimental studies in wistar rats, the control group with LPS intrasisternal and intranasal placebo ($n = 18$), the experimental group with LPS and ACTH$_{4-10}$ intranasally ($n = 18$). The variables are PGE2 and IL-1b levels from CSF were analyzed statistically. The results showed that the levels of IL-1b and PGE$_2$ significantly different between the control group and the experimental group ($p <0.05$). Conclusion, administration ACTH$_{4-10}$ Synthetic shown to decrease IL-1β and PGE$_2$ levels in CSF significantly in animal model that induced by LPS*

Keywords: *ACTH$_{4-10}$ synthetic, PGE$_2$, IL-1β, meningitis, LPS*

I. INTRODUCTION

Bacterial meningitis is a life-threatening disease because the mortality and morbidity remains high (van de Beek *et al.,* 2006) until now although the new antibacterial drugs was applied (Dhamija and Bansal, 2006). In the relation with this problems, the biomolecular research in pathophysiology of bacterial meningitis by using live bacteria or bacterial products (LPS, Peptidoglycan or bacterial DNA) were performed with the aims to searching the factors as the central role in the brain damage that caused morbidity and mortality (Nau and Bruck, 2002). The results of this studies revealed that both gram positive or gram negative bacteria and its products were able to trigger the inflammatory response that consist of increasing production of proinflammatory and anti-inflammatory mediators such as TNFα, IL-1β, IFNγ, PAF and PGE$_2$ from the resident cells in the brain that caused increased permeability of BBB, with the leukocyte influx as a consequence to arachnoid space resulting in vasospasm, arteritis and intravascular thrombosis and eventually led to global cerebral ischemia (Bucki *et al.,* 2007; Katchanov *et al*., 2010).

Prostaglandin is produced during neuro inflammation disease and the production of PGE$_2$ level increase by COX-2 and able to mediate the inflammatory response such as vasodilatation, increased vascular permeability and leukocyte rolling to luminal side of cerebral micro vascular and influx leukocyte (Hewett et al., 2006). At the first time, the aim of PGE2 released is related to host defense mechanism to increasing the leukocyte to eradicate the bacteria, but the excessive production of PGE$_2$ may harmful because may lead the continuous inflammatory processes in the CNS (Hewett *et al.,* 2006).

According with COX-2 activity in neuro inflammation, COX-2 inhibition effectively inhibit increasing BBBP and reorganization of cytoskeleton endothelial cells cerebral micro vascular induced by TNFα (Mark *et al.,* 2001), but the other hand, the inhibitor COX-2 may lead increased Nitric Oxide concentration significantly and causing BBBP disruption (Boje *et al.,* 2003). In the relation with this fact, need to find the new approach therapeutic that able to reduce the PGE2 level but not influence the NO level that to prevent the brain damage.

In relation to cerebral ischemia that occurs as a result of cerebral microvascular inflammatory response in patients with bacterial meningitis, currently some research in vivo and in vitro in animals and humans with cerebral ischemia showed clinical improvement with administration of analog $ACTH_{4-10}$ ($ACTH_{4-7}$ Pro -Pro-Gly) synthetic (Fadiukova *et al.,* 2001). The research results that favorable effects consist of angio protection, anti-hypoxic and neurotropic. The aim of this study is to determine effects of $ACTH_{4-10}$ synthetic in LPS-induce meningitis in PGE_2 and IL-1β levels in CSF.

II. RESEARCH METHODS

Material **:** Elisa kit for PGE_2 were purchace from R&D, inc manufactured and distributor Minneapolis, USA with catalog number KGE004B SKGE004B PKGE004B, IL-1β were purchace from eBioscience North America with catalog number BMS630/BMS630TEN, Lipopolysaccaride (LPS) from Escherichia Coli serotype 026:B6 were purchace from Sigma-Aldrich Inc (St Louis, MO) and ACTH4-10 synthetic 1% were purchace from Semax International Inc, Moscow-Russia.

Assesment PGE_2 and IL-1β level in CSF : 50µl CSS from the cisternal puncture was diluted and the examination conducted with accordance with standart procedure from the manufactured instructions and the PGE2 and IL-1β concentrations is stated in pg/mL.

Challenge LPS : After disinfection protocols, the rat was handle and fixed under local anesthesia with Ketamin (Park-Davis Co., Morris Plains, NJ) (7mg/kg) intravenously. Cisternal puncture performed using 32-gauge needle connected to a syringe on the external occipital crest opistion & side basion superior & inferior side. The needle is inserted at 1/3 distance between opistion and basion in the quadrilateral formed by the superior and inferior. At the time of injection, if the CSF was visible, it means that the needle is in the right position and after removing 50µl CSF, LPS 20ng can be inserted.

$ACTH_{4-1}0$ synthetic administration : the wistar rats were handle and the $ACTH_{4-10}$ synthetic 1 drops (50µg) were administrated intranasal with the micropipettes for each nostril

Replication and randomized samples : This study we used an animal model, adult Wistar rats, male, at 2 weeks of age with bodyweight 150-200g. The inclusion criteria was the healthy rats that are specified with active movements and normal eating and drinking. According with the Federer's formula, the total samples were 36 rats and with completely randomized design, these samples were dividing into 2 groups with 18 rats respectively. In the first group as the control group with LPS intracisternal and Placebo (solvent formula of $ACTH_{4-10}$ synthetic) intranasal 2 hour after LPS; and the second as an experimental group with LPS intracisternal and $ACTH_{4-10}$ synthetic intranasal.

Experimental methods : 4 hours after LPS inoculation, CSF was sampled for determined the level of PGE_2 and IL-1β. After that, the placebo and $ACTH_{4-10}$ synthetic were administered and the rats were observed for 2 hours. CSF sample was taken at either 2 hours after administering $ACTH_{4-10}$ synthetic or placebo and IL-1β level and PGE2 level were determined again.

Analysis. Comparison between the control and experimental group parameter was analyzed by using descriptive analysis and inferential analysis. Significantly if p<0,05. Spearman correlation analysis study using to evaluate the correlation between the parameters in this study, with significance if p<0,05

III. RESULTS

The level of PGE_2 in CSF

The level of Prostaglandin was examined quantitatively by using ELISA method. The data for the PGE_2 level in the CSF for experimental group was not normally distributed (Kolmogorov-Smirnov test, p< 0.05), thus the analysis by using Kruskal-Wallis. This study revealed the significant differentiation of PGE_2 level between the control and experimental group (Table 1)

Table 1. Effects of ACTH4-10 on PGE2 level in CSF

Group	n	The level of PGE2					Brown-Forsythe
		x	SD	Median	Min	Max	
Normal Rats	**18**	**26,915**	**9,576**	**24,056^a**	**13,146**	**43,732**	
LPS	**18**	**413,947**	**767,292**	**155,248^b**	**56,344**	**3311,569**	**p=0,000***
$ACTH_{4-10}$	**18**	**31,180**	**16,149**	**24,056^a**	**13,232**	**73,066**	

Note : *significance at α=0,05

[a,b] the different superscript revealed that the differences between the groups in this study (Wilcoxon-Mann Whitney test)

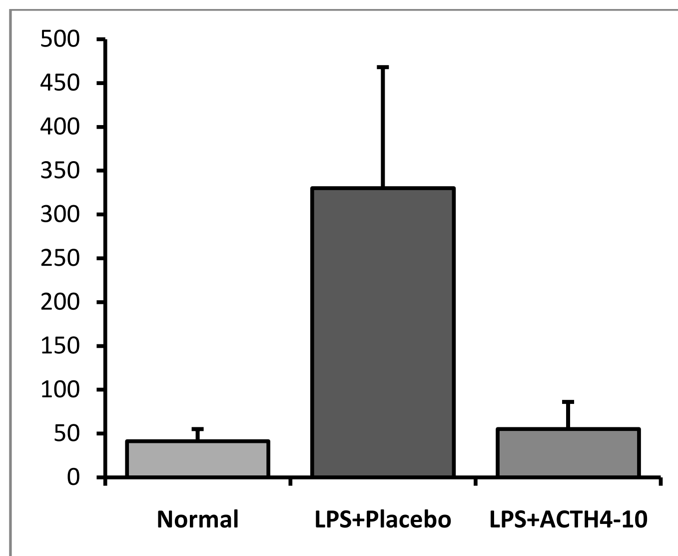

Figure 1. The comparison of PGE$_2$ level in the CSF between normal, control and experimental groups

The level of IL-1β in the CSF

In this study, qualitative measurement of IL-1β was determine by using ELISA method. The data for each groups was normally distributed (Kolmogorov-Smirnov test, p>0,05), but data variance between groups inhomogeneous (Levene's test, p<0,05), so the analysis in this section using Brown-Forsythe (Table 2). The results showed that there were significant differences (p<0,05) between the LPS and ACTH$_{4-10}$ groups, and the administration of ACTH$_{4-10}$ synthetic caused the IL-1β level return to nearly normal level.

Table 2. Effects of ACTH$_{4-10}$ synthetic on IL-1β level in CSF

Group	n	IL-1β				Brown-Forsythe
		x	SD	Min	Max	
Normal	**18**	**85,610[a]**	**3,254**	**82,197**	**96,882**	
LPS	**18**	**151,893[c]**	**52,675**	**104,355**	**279,162**	**p=0,001***
ACTH$_{4-10}$	**18**	**90,933[b]**	**5,498**	**83,880**	**103,187**	

Note *significance at α=0,05

[a,b,c] the different superscript indicates the differences between groups (multiple comparison test from Games-Howell)

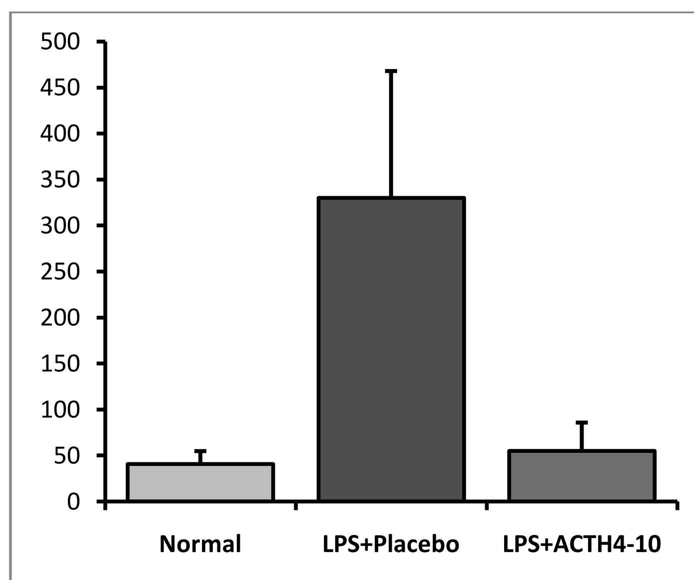

Figure 2. The graphic of average of IL-1β level between normal, LPS and ACTH$_{4-10}$ synthetic

Correlation analysis Spearman : The correlation ratio PGE_2 to IL-1β was 0.739 with significance p=0.000. it means that increased PGE_2 have the strong correlation with increased level of IL-1β

IV. DISCUSSION

Interleukin-1β (IL-1β) is produced by several cells such as monocytes, macrophages, granulocytes, endothelial, microglia and astrocytes cells. In the inflammation situations, LPS or live bacteria could stimulate the IL-1β production from the resident cells in the brain. This cytokine was capable to stimulate the production of another cytokines such as IL-6 and TNFα, and increased expression of adhesion molecules resulting in increased adhesion and transport neutrophil and monocytes cells across the blood-brain barrier (Dinarello, 2005).

The mechanism of increased IL-1β is associated with activation of Casp-1 as the central role in the formation of mature IL-1β (Los et al., 1999). In bacterial meningitis, Casp-1 mRNA and protein expression increased and this is associated with increased levels of IL-1β. Signaling pathway Casp-1-IL-1β plays an important role in the induction and amplification of the host inflammatory response during bacterial meningitis. Depletion of Casp-1β gene could decrease IL-1β induction and reduction of NFkB activity significantly (Scheld et al., 2002). In connection with the above mechanism, the role $ACTH_{4\text{-}10}$ synthetic in Casp-1 activation in cerebral inflammation remains unclear. From the literature study, only found 1 results in patients with arthritis who stated that αMSH were identified with synthetic $ACTH_{4\text{-}10}$ did not affect the activation and secretion of Casp-1 directly (Capsoni et al., 2009). Accordingly, $ACTH_{4\text{-}10}$ synthetic capabilities to directly suppressing the production of IL-1β through Casp-1 activity remain unclear and the further research needed to fulfill the explanation this mechanism.

In relation with the mechanism of production of IL-1β was associated with activation of NFkB, a drug that capable to suppress this activation of NFkB is expected to control the excessive inflammatory response that can potentially damage brain cells (Koedel et al., 2000). This is consistent with the results of this study, administration of synthetic $ACTH_{4\text{-}10}$ significantly decreased the levels of IL-1β in the CSS. The reduction mechanism is suggestion related to the ability $ACTH_{4\text{-}10}$ synthetic inhibit NFkB activation in the cells within the CNS (Gusev and Skvortsova, 2003).

In bacterial meningitis, endotoxin (LPS) stimulates the expression of COX-1 from neurons, astrocytes and microglia, to effect an increase in the synthesis of PGE_2. PGE_2 levels in the CSS maximum is reached after 6-8 hours post insertion LPS intracisternally (Jaworowicz *et al*., 1998; Wu *et al*., 2006). These results are in accordance with the results of this study, in the control group with LPS showed PGE_2 level increased significantly ($p < 0.01$) to compared with normal rats. PGE_2 synthesis prepared by COX-1/COX-2/membrane-associated cPGES (cPGES-m) and nuclear / perinuclear COX-2/mPGES-1/cPGES (Vazquez-Tello *et al*., 2004). In connection with this, LPS was able to induce the synthesis of PGE_2 by increasing regulation of the expression of COX-2 and mPGES-1 (Ikeda-Matsuo *et al*., 2005).

In relation with NFkB activity, mechanism of increased regulation of PGE_2 production associated with NFkB because this transcription factor could regulate COX-2. NFkB is activated through complex pathways centered on P38 kinase, JNK and ERK1 / 2 MAPK. Some studies have found that induction of COX-2 expression requires activation of NFkB and binds at the promoter region of COX-2 (N'guessan *et al*., 2007; N'guessan *et al*., 2006; Chun and Surh, 2004; Gong *et al*., 2008). Thus, inhibition of COX-2 enzyme activity or signaling pathways that regulate the transcription of COX-2 can be used as a mechanism to suppress the inflammatory response mediated by COX-2.

LPS stimulates microglia cells induces PGE_2 synthesis through MAPK signaling pathway 3: JNK, ERK, and p38. JNK and p38 signaling pathway regulates the production of PGE2 via the JNK-AP-1-dependent and p38-NFkB, whereas phosphorylation of p38 using phospholipase A2 (PLA2) to release acid catalysis arachidonik (You *et al*. 2005). ERK signaling pathways regulate the synthesis of PGE2 by activating a transcription factor that can induce the expression elk1, other transcription factors c-Fos bound to the AP-1 (Gong *et al*., 2008). Regarding the signaling pathways mentioned above, the mechanism $ACTH_{4\text{-}10}$ synthetic in suppressing PGE_2 synthesis presumably relates to the ability of $ACTH_{4\text{-}10}$ to inhibit transcription factor NFkB (Gusev and Skvortsova, 2003). This is consistent with the results of this study that decreased levels of PGE_2 in experimental group with synthetic $ACTH_{4\text{-}10}$ intranasally.

Another mechanism associated with the synthesis of PGE_2 by NO that potential in modulating the expression of COX-2. It is based on the previous results study that found decreased PGE_2 synthesis by administering NOS inhibitors. Research on the effect of NO on COX activity (Marnett *et al*., 2000) suggests that the signaling pathway of iNOS induction by co-expression cytokines and COX-2 very closely linked. The mechanism of induction of PGE_2 by NO occurs at the level of transcription and expression of genes that induce COX-2 (Ling *et al*., 2005). With respect to the mechanism of induction of PGE_2 by NO, $ACTH_{4\text{-}10}$ was proven to inhibit exogenous NO production via inhibition of NFkB activation (Gusev and Skvortsova, 2003). Previous

studies carried out by Chiao et al are using αMSH in liver cells stimulated with LPS also get similar results (Chiao *et al*., 1996). Similarly, the results of this study also showed similar results.

From the Spearman correlation test in this study shows that there is a significant correlation between IL-1β to increase PGE_2 in CSS. This is consistent with previous studies that found that IL-1β was able to induce PGE_2 synthesis through activation of COX-2 mRNA (Neeb *et al*., 2011).

V. CONCLUSION

$ACTH_{4-10}$ synthetic is proved to decrease PGE_2 and IL-1β levels in the CSF rats induced by LPS.

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