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 α-MSH 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-1β 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 PGE$_2$ and IL-1β levels from CSF were analyzed statistically. The results showed that the levels of IL-1β 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_{4-10}(Pro-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 purchase from R&D, inc manufactured and distributor Minneapolis, USA with catalog number KGE004B SKGE004B PKGE004B, IL-1β were purchase from eBioscience North America with catalog number BMS630/BMS630TEN, Lipopolysaccaride (LPS) from Escherichia Coli serotype 026:B6 were purchase from Sigma-Aldrich Inc (St Louis, MO) and ACTH4-10 synthetic 1% were purchase from Semax International Inc, Moscow-Russia.

**Assessment 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 PGE_{2} 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_{4-10} 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 administrated 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 PGE_{2} 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>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>The level of PGE_{2}</th>
<th>Brown-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x</td>
<td>SD</td>
</tr>
<tr>
<td>Normal Rats</td>
<td>18</td>
<td>26,915</td>
<td>9,576</td>
</tr>
<tr>
<td>LPS</td>
<td>18</td>
<td>413,947</td>
<td>767,292</td>
</tr>
<tr>
<td>ACTH_{4-10}</td>
<td>18</td>
<td>31,180</td>
<td>16,149</td>
</tr>
</tbody>
</table>

Note: *Significance at α=0.05

\textsuperscript{a,b} the different superscript revealed that the differences between the groups in this study (Wilcoxon-Mann Whitney test)

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**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 ACTH₄₋₁₀ groups, and the administration of ACTH₄₋₁₀ synthetic caused the IL-1β level return to nearly normal level.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>IL-1β</th>
<th>Brown-Forsythe</th>
<th>x</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>18</td>
<td>85.610a</td>
<td></td>
<td>3,254</td>
<td>82,197</td>
<td>96,882</td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td>18</td>
<td>151.893c</td>
<td></td>
<td>52,675</td>
<td>104,355</td>
<td>279,162</td>
<td>p=0.001*</td>
</tr>
<tr>
<td>ACTH₄₋₁₀</td>
<td>18</td>
<td>90.933b</td>
<td></td>
<td>3,498</td>
<td>83,880</td>
<td>103,187</td>
<td></td>
</tr>
</tbody>
</table>

**Note** *significance at α=0.05*

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

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**Figure 1. The comparison of PGE₂ level in the CSF between normal, control and experimental groups**

**Table 2. Effects of ACTH₄₋₁₀ synthetic on IL-1β level in CSF**

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**Figure 2. The graphic of average of IL-1β level between normal, LPS and ACTH₄₋₁₀ synthetic**
Correlation analysis Spearman: The correlation ratio PGE2 to IL-1β was 0.739 with significance p=0.000. It means that increased PGE2 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-1 gene could decrease IL-1β induction and reduction of NFkB activity significantly (Scheld et al., 2002). In connection with the above mechanism, the role ACTH1-10 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 ACTH1-10 did not affect the activation and secretion of Casp-1 directly (Capsoni et al., 2009). Accordingly, ACTH1-10 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 ACTH1-10 significantly decreased the levels of IL-1β in the CSS. The reduction mechanism is suggestion related to the ability ACTH1-10 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 PGE2. PGE2 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 PGE2 level increased significantly (p <0.01) to compared with normal rats. PGE2 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 PGE2 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 PGE2 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 PGE2 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 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 ACTH1-10/Sythetic in suppressing PGE2 synthesis presumably relates to the ability of ACTH1-10 to inhibit transcription factor NFkB (Gusev and Skvortsova, 2003). This is consistent with the results of this study that decreased levels of PGE2 in experimental group with synthetic ACTH1-10 intranasally.

Another mechanism associated with the synthesis of PGE2 by NO that potential in modulating the expression of COX-2. It is based on the previous results study that found decreased PGE2 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 PGE2 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 PGE2 by NO, ACTH1-10 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 PGE2 in CSF. This is consistent with previous studies that found that IL-1β was able to induce PGE2 synthesis through activation of COX-2 mRNA (Neeb et al., 2011).

V. CONCLUSION

ACTH$_{4-10}$ synthetic is proved to decrease PGE2 and IL-1β levels in the CSF rats induced by LPS.

REFERENCES