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Anti-inflammatory activity of Corticosteroids and Vitamin C Combination in HUVECs culture Sepsis Model

Purwoko[1]*, HMS Chandra Kusuma[2], Nur Permatasari[3], Mohammad Aris Widodo[3]

[1]Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia
[2]Laboratory of Pediatric, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia
[3]Laboratory of Pharmacology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

Abstract : Sepsis is a serious health problem with high morbidity and mortality. The main factor deciding the final outcome in sepsis is an inflammatory response, not a bacterial infection. The purpose of this study was to evaluate the combination of corticosteroids and vitamin C in HUVECs sepsis conditions to reduce the inflammatory response of TNF-α and IL-1β. Sepsis conditions obtained by co-culture 3 treatments group of HUVECs cell and monocytes (THP1), stimulated with 10 ng/ml LPS for 3 hours. The concentration of TNF-α and IL-1β were analysed using ELISA at 450 nm wavelength. The results showed that TNF-α on the second, fourth and eighth hours of the test is significant differences in the levels of TNF-α between research groups. Results IL-1β at 2 and 8 hours is also significant differences between the levels of IL-1β research groups. The declines in the levels of IL-1β and TNF-α are those of methylprednisolon10 μM. The combination of corticosteroids and vitamin C protection against endothelial effects through suppressing the inflammatory response due to infection which proved to decreased levels of pro-inflammatory cytokines which in this case is TNF-α and IL-1β.
Keywords: anti-inflammation, Corticosteroids, Vitamin C, Sepsis.

Introduction

Sepsis is a health problem by high mortality and morbidity. In Europe ± 150,000 patients with sepsis, severe sepsis (with multi-organ dysfunction) and septic shock per year, of which 65,000 of them are dead[1]. At least> 50% of the deaths were due to septic shock and organ failure. The cause of death from sepsis is not due to infections, but the inflammatory response to the infection[2].

Microvascular endothelial cells become activated in sepsis and lead to an increase in the inflammatory response. To support this hypothesis, it has been shown that septic stimuli (LPS, TNF-α) started the activation of transcription factors such as NFκβ and AP-1, resulting in transcriptional activation of multiple genes. This causes the release of pro-inflammatory cytokines (eg, TNF-α, IL-1β, etc), and increased expression of adhesion molecules (eg, E-selectin, ICAM-1, VCAM-1) and chemokines by endothelial cells [3,4,5,6].

Cortisol inhibits the transcription of genes encoding pro-inflammatory cytokines by reducing the activity of NF-κβ, by binding to the glucocorticoid receptor and binds to p65 as a result of corticosteroids would inhibit the synthesis or action of the majority of pro-inflammatory cytokines. Although the anti-inflammatory

effects of corticosteroids are mainly caused by compression synthesis of pro-inflammatory cytokines, in part also due to the effect of increased production of anti-inflammatory cytokines such as IL-10. Glucocorticoids initiate a shift from Th2 response to a Th1 response which will increase the production of IL-4, IL-10 and IL-13[7,8].

Vitamin C is an antioxidant group is soluble in water, which can reduce the negative impact of oxidants, including enzymes and proteins binding metals. The function of antioxidants is to prevent the formation of hydroxyl radicals, break the chain reaction of the oxidant, and reduce oxidants into other less reactive substances like H2O and O2, inhibiting lipid peroxidation and direct scavenger of ROS.

Vitamin C works in KKI (Kappa Kinase Inhibitor) is in an active state so that the bond Iκβ phosphorylate with NF-κβ. Thus, NFκβ become active. By inhibiting KKI, then phosphorylation will also be inhibited so that the NF-κβ becomes inactive, then the production of pro-inflammatory cytokines would be decreased[9].

This study using HUVECs sepsis model and control the administration of low doses of corticosteroids and vitamin C. The aim of this study was to evaluate the combination of corticosteroids and vitamin C in cultured HUVECs in sepsis conditions to reduce the inflammatory response of tumour necrosis factor alpha (TNF-α) and interleukin-1 (IL-1β).

Materials and Methods

Umbilicus Sample Collection

Umbilicus obtained from the caesarean section that meets either inclusion or exclusion criteria, i.e.: pregnant women with a healthy weight and normal height, HB> 11 healthy babies weighing more than 2.5 kg, Apgar score of 7-9 2), the mothers suffering from hypertension and a history of diabetes mellitus, premature rupture of membranes for more than 6 hours, and fetal distress. Umbilicus must be approximately 15 cm straight long and not threaded. Umbilicus obtained from childbirth cut and inserted into the cord solution comprising a solution of Hank's Balanced Salt Solution (#H4641; Sigma-Aldrich, USA), penicillin-streptomycin 1% (#P0781; Sigma-Aldrich, USA), Hepes 25 mM and NaHCO3 20 mM without rinsing, and stored at 4°C during transport process.

HUVECs Isolation and Culture

Culture process was performed in Biosafety Cabinet Level 2 (Safe Fast Elite 212D, Faster, Italy). The cell cultures subsequently incubated in an incubator (37°C, 5% CO2) until reached 80% - 90% confluent. Flow cytometry analysis was conducted to identify the endothelial cells using a specific marker of the endothelial cell line, VEGF. The HUVECs purity percentage is 99.7%.

Monocyte Cell Line Culture and Stimulation by LPS

Monocyte cell line THP-1 (#TIB202; ATCC, USA) was cultured in incubator (37°C, 5% CO2) until reached population 8 x 10^5 cells/ml. Cells were re-suspended with sterile complete medium contained 10 ng/ml LPS from *Pseudomonas aeruginosa* 10 (#L9143; Sigma-Aldrich, USA). Cells viability was counted by haemocytometer. Cells were grown in 24-well culture plate with the number of cells 1x10^6 cells/ml per well and incubated for 3 hours (37°C, 5% CO2) to produce conditioned medium. After 3 hours, the culture medium was collected and stored at -70°C.

Combination Treatment of Steroid and Vitamin C and TNF-α and IL-1β Concentration Measuring

The matured HUVECs cell culture medium were withdrawn, and washed with SFM sterile as much as 1X, then exposed to conditioned medium with 3 kinds of treatment variations, i.e.: a) methylprednisolone (# 4819; Tocris, UK) with variations in concentrations of 10, 20 and 40 μM; b) vitamin C / L-Ascorbic Acid (# A4403; Sigma-Aldrich, USA) with various concentrations of 50, 75 and 100 μM and c) a combination of steroid and vitamin C (methylprednisolone 10 μM + vitamin C 50 μM; methylprednisolone 20 μM - vitamin C 75 μM and 40 μM methylprednisolone + vitamin C 100 μM) and incubated with time variations of 2, 4 and 8 hours. HUVECs cell culture without treatment act as controls. After incubation was complete, the culture

medium of each treatment period was collected to do ELISA assay to measure the concentrations of TNF-α and IL-1β.

Statistical Analysis

Data were analysed using descriptive and analytical tests in SPSS Program. ANOVA and T-test were performed to measure the significant different of cytokines in each group ($p < 0.05$).

Result and Discussion

The results showed that the expression of TNF-α on the second, fourth and eighth hour of the test were simultaneously different at $p = 0.000$ ($p < 0.05$). This means that there are significant differences in the levels of TNF-α between the study groups (Figure 1). With these results, it can be assumed that both corticosteroids and vitamin C may suppress the expression of TNF-α which in turn prevents further inflammatory processes. Although the effect of corticosteroids and vitamin C combination is different in each hour of observation.

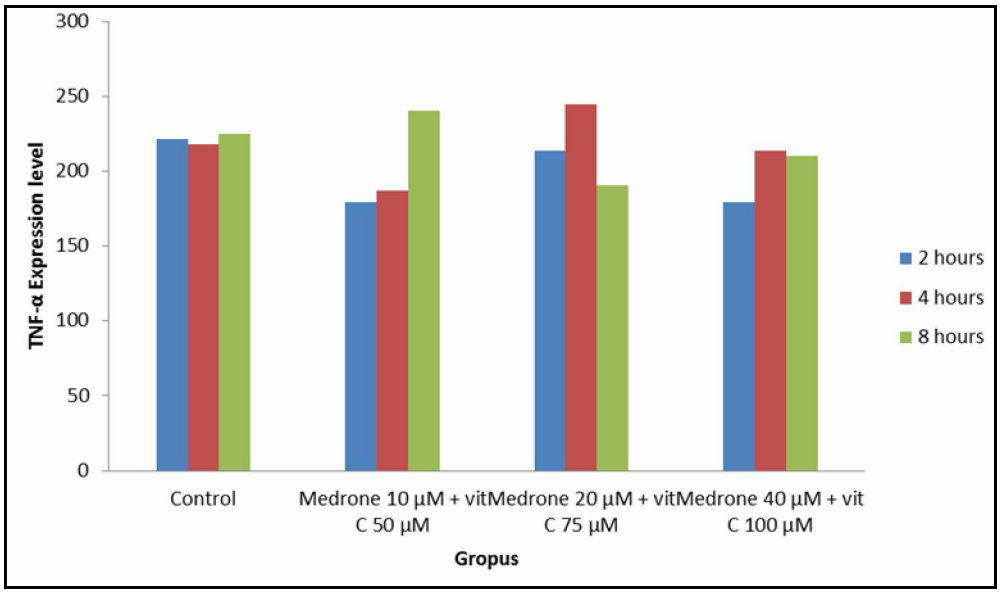

Figure 1: The comparison of TNF-α levels changes in each treatment group.

Similar to the expression of TNF-α, IL-1β is also a pro-inflammatory cytokine involved in the inflammatory process and sepsis. In general, the results of this study suggest that corticosteroids and vitamin C suppress the expression of IL-1β and preventing further inflammation. But the treatment gourp in the 2 hours and 8 hours showed that levels of IL-1β was increase. This is likely caused by the endothelium that is becoming more intact because it was given vitamin C, so IL-1β expression was derived from endothelial will increase, it does not happen on TNF-α expression was derived not from the endothelium (Figure 2).

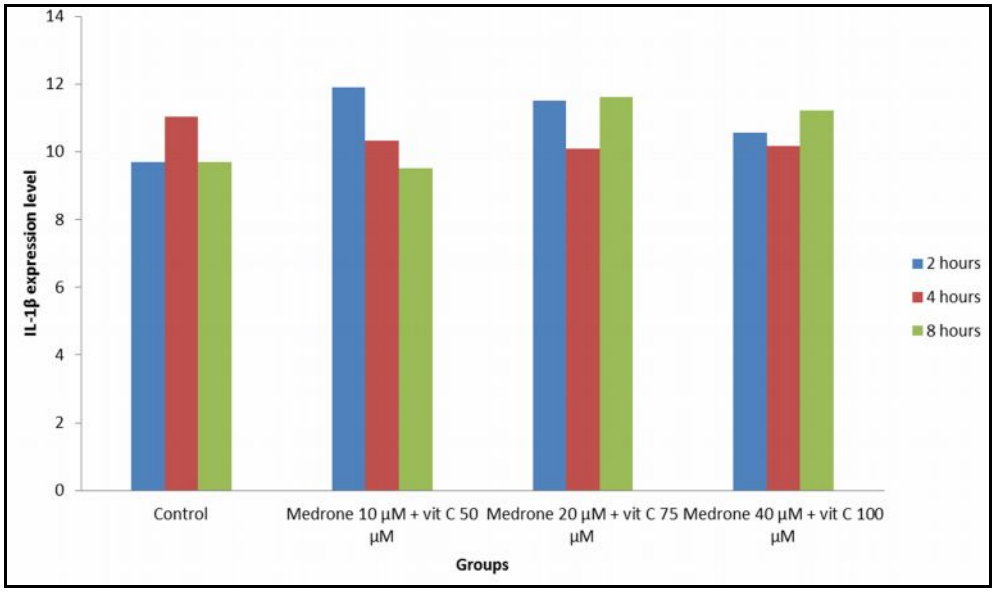

Figure 2. The comparison of IL-1β levels changes in each treatment group

The results of this study prove that corticosteroid and vitamin C combination can provide protection against endothelial effects, through suppressing the inflammatory response due to infection. The combination of methylprednisolone and vitamin C can suppress the expression of TNF-α so that the inflammatory process is inhibited, despite an increase in IL-1β which may be caused by a more protected endothelial cells.

IL-1β is a central mediator that triggers immunity and inflammation. In the family of IL-1β are seven ligands with the activity of agonists (IL-1α and β, IL-18, IL-33, IL-36α, β, γ), 3 receptor antagonist (IL-1Ra, IL-36Ra, IL-38) and anti-inflammatory cytokines (IL-37). Setting strict receptor antagonists, receptor feeder (decoy) and inhibitory signals ensure a balance of immunity and inflammation is uncontrolled. Moreover, IL-1β play a role in influencing lymphoid cells that play a role in immunity. IL-1 directly affects all the cells and organs and is a pathogenic mediator of autoinflammatory, autoimmune, infectious and degenerative diseases. The effects of IL-1β in the central nervous system including fever (endogenous pyrogens) and the activation of the hypothalamic-pituitary-adrenal (HPA). At the elevated temperatures increased leukocyte migration. Cortisol is part of HPA axis that regulate immune function and inflammatory[2,10,11].

This Study shown for the first time that physiologically relevant vitamins, vitamin C, inhibit activation of NF-κB by a variety of stimuli in endothelial cells. Inhibition of cell types that are found as independent and activating stimulus, because the pathways that include TNF, PMA, and H2O2, in the transformation and primary endothelial cells, and other cell types (T cell line and cell astrocytoma, not shown) proved sensitive to vitamin C. other groups have reported that in Jurkat cells, vitamin C actually increases the activation of NF-κB-mediated TNF-α[12,13,14].

Conclusion

The combination of corticosteroids and vitamin C provide protection effects on endothelial by suppressing the inflammatory response during the infection process. Vitamin C and methylprednisolone combination suppress the expression of TNF-α so that the inflammatory process is inhibited, despite an increase in IL-1β which may be caused by more protected endothelial cells.

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