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Phenyl butyrate acid inhibit tnf-alpha-induced nuclear atf6 expression in endothelial cells

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Abstract: *Endothelial cell (EC) is important tissue that has high plasticity in response to vascular millieu change. However, its plasticity turn EC lead to its dysfunction and contribute in several disease patomechanism. An inflammation agent such as TNF-α can induce EC dysfunction via endoplasmic reticulum stress (ERS) pathway. Targeting ERS was new approach in vascular biology to inhibit EC dysfunction. One of its biomarker is activating transcription factor 6 (ATF6). However, ERS role in endothelial cells is still poorly understood. Hence we performed in vitro experiment using phenyl butyrate acid as ERS inhibitor to TNF-α -induced-ATF6 expression in human vein derived endothelial cells. We measured ATF6 expression in endothelial cells as ERS biomarker and use phenyl butyrate acid (PBA) as potent selective ERS inhibitor to block its pathway. The early result shows that PBA decrease translocated ATF6 expression in endothelial culture. From the result, it can been concluded that PBA has role in decrease endoplasmic reticulum stress in endothelial cells.*

Keywords: *Endothelial cell, patomechanism, phenyl butyrate acid*

INTRODUCTION

For a last decade, researcher has been agreed that endothelial cell (EC) is the strong gate of cardiovascular health. EC become a popular study in cardiovascular research for it was considered as most largest body organ covered the whole internal surface blood vasculature. Good quality of the blood vessel wall was determined by quality of endothelial cell. Metabolic perturbance in endothelial cells will lead its dysfunction called endothelial dysfunction (ED).

It has been well known that chronic inflamation was main cause of ED and it has many manifestation. EC has dynamic properties and has high plasticity respond to microenvirontment change (Dejana, Hirschi et al. 2017). Biochemical and hemodynamic changes of blood flow requires a high adaptive endothelial cells in order to maintain homeostasis of blood flow. Physiologically, this plasticity was required in the process of normal growth and development of early life embriogenic formation(Krenning, Barauna et al. 2016). However, in certain pathologic process, this plasticity lead to phenotipe shift of normal EC to contribute in ED.

TNF-α has been well-known as a potent pro-inflamatory cytokine. As pleiotropic cytokine, it has been known that TNF-α had important role in chronic inflamation that cause ED. In endothelial cells, stimulation of TNF-α as proinflammatory cytokines will stimulate its phenotype shift to be osteogenic lineage.

On the other hand, a cellular level mechanisms that emerged recently and became more attractive research area is what is known as the endoplasmic reticulum stress (ERS). ERS define as endoplasmic reticulum (ER) disfunction due to ER inability increasing its capacity in the folding process of the protein. However, ERS in endothelial cells is still poorly understood. Base on this, it was interesting to dig deeper the role of ERS in inflamation of endothelial cells. We hypothesized that ERS processes underlying the

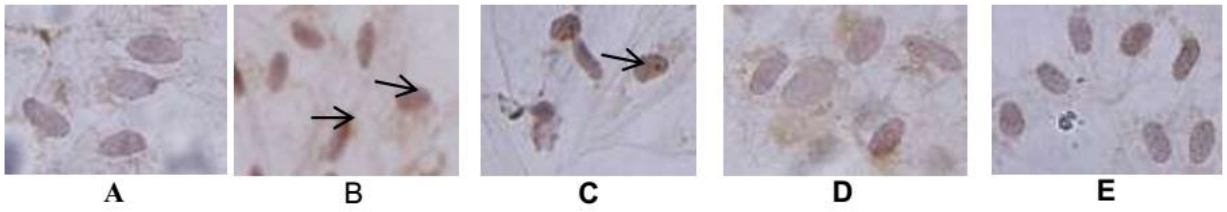
process of the changing nature of endothelial cells towards osteoinductive. It is hoped this knowledge will fill knowledge gap to inhibit the processes of advanced due to the KV

MATERIALS AND METHODS

The study ethical issue was approved by Human Ethical Comitte University of Brawijaya, Malang, Indonesia (141/EC/KEPK/S3/05/2016). Umbilical cord was taken from baby born with parents permission. Endothelial cells were obtain from baby born umbilical vein which was isolated by 0.05% collagenase (from Clostridium histolyticum, Worthington, Lakewood New Jersey, USA) digestion of umbilical cord veins for 15 minutes. Blood components and non-adherent cells were removed by regularly medium change. Obtained cells then plated into 25 cm2 Falcon flasks in medium RPMI-1640 plus 25 mM HEPES and L-glutamine, penicillin 100 U/ml, streptomycin 100 mg/ml, 10% heat-inactivated fetal calf serum (FCS) and 10% new-born calf serum (NBCS). Cells then allowed to attach its dish for 3 hours. At confluence, EC were detached with 0.05% trypsin/0.02% EDTA, and sub-cultured at ratio 1:3 in the above EC cell growth medium containing, in addition, 15 mg/ml growth supplement (Sigma Chemical Co., UK) and heparin 50 U/ml (Leo Laboratories Limited, UK). Cells then plated on 24 well plate cell culture dishes (Falcon;BD Biosciences, New Jersey) in M199 medium with 20% Fetal Bovine Serum, 100 lg/ml pen-strep, 0.1 mg/ml heparin, and 0.05 lg/ml EGF. Cells culture then incubated at 37^{0}C in a 5% CO2 incubator with humidified. After 80% confluency, cells were exposed with numeral dose of TNF-α 5 ng/ml and 4-PBA 1,2 and 3 nM/L in eight hours. Cells culture then fixed using methanol 5 % and immunostained with anti-ATF-6 antibody (Bioss, Beijing, China). Data was analyzed by one-way ANOVA and the difference between groups was analyzed by post hoc LSD comparison test. Data were presented as mean ± standard error of the mean (SEM). p-values considered significant less than 0.05 statistically.

RESULTS

We show in this study that TNF-α increase endothelial nuclear ATF6 expression in positive control and minimally expressed in negative control. As shown in Fig 1, PBA treatment has decrease ATF6 in dose dependently significantly. As seen in immunocitochemisty results, there are much of positive ATF6 stained cells in control positive that show decrease gradually after PBA treament. The minimally ATF6 expression is shown in PBA 3 mM/L and not significant statistically compared to negative control without TNF-α treatment



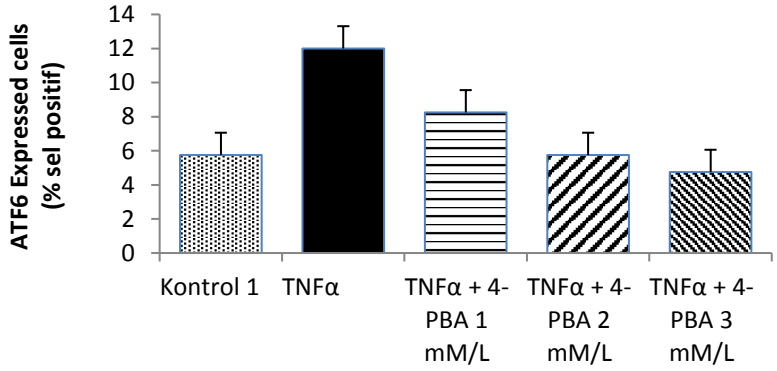


Fig 1. Immunostain nuclear ATF6. Negative control (A) and positive control (B) has been shown different result of ATF-6 expression. PBA treatment at dose 1 nM/L (C) ATF6 dominantly compare to TNFα+2 nM/L (D) group and TNFα+3 nM/L group (E) (magn 400X)

In this study, from several ERS mediators, we took ATF6, to see its role in modifying these changes. PBA is used as a selective and potent inhibitor of the ERS pathway (Zhang, Nakajima et al. 2013). TNF-α was used with concentrations of 5 ng / ml for 8 hours exposure time to induce these changes (Illiandri, Sujuti et al. 2016). The mode of action PBA is a chemical chaperon that assists the biological chaperones in the RE lumen (Xiao, Giacca et al. 2011). 4-PBA is a short chain fatty acid that has long been used as ammonia scavenger in urea cyclic disorder (urea cyclic disorder). As a chemical chaperon, 4-PBA works by reversing the process of mislokalization and protein aggregation that occurs in some diseases (Perlmutter 2002). In other words, the PBA acts as an adjunct agent of the biological chaperon molecules present in the RE lumen.

As indicated by the above results, administration of PBA decreases the expression of ATF6 nuclei at doses of 1, 2 and 3 mM / L. This corresponds to the results obtained by Zhang showing that PBA has the effect of lowering ATF6 expression although this report is still limited to renal epithelial cells from rats (Zhang, Nakajima et al. 2013)

Inhibition of ERS is reported to have an inhibitory effect on the pathomechanism of cardiovascular disease especially in the hardening of large blood vessels (Spitler, Matsumoto et al. 2013, Spitler and Webb 2014) In the process of atherosclerosis, ERS also can not be underestimated again its role in initiating the occurrence of disease (Ivanova and Orekhov 2016). Nevertheless, as far as our knowledge, no study has been conducted before concerning osteoinductive shift endothelial cell related in endoplasmic reticulum stress. Although these results are still based on a small portion of the ERS markers, at least this has provided a new horizon of ERS involvement in the process of changing the osteoinductive properties of endothelial cells.

CONCLUSION

PBA decrease ATF6 expression dose dependantly. From the result, it has been concluded that ERS mediates osteoinductive phenotype shifting in inflammation endothelial cells.

CONFLICT OF INTEREST

The authors declare that this research have no conflict of interest.

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