

Original article**The role of 4-pba on tnf-alpha related apoptosis on human vein endothelial cells**

Oski Illiandri

Abstract

Tumor Necrotizing Factor-alpha (TNF- α) has been well-known as a potent pro-inflammatory cytokine involved in many degenerative disease. One of its primary target organ-damaged was endothelial cells which will lead to its endoplasmic reticulum stress (ERS) and induced its apoptosis. Endothelial cell apoptosis will lead high implication in many disease pathomechanism of many degenerative disease. Some study report ERS inhibitor 4-Phenyl Butyric Acid (4-PBA) properties to inhibit inflammation process in endothelial cells. However, whether 4-PBA can decrease apoptosis level in inflammation of endothelial cells is still poorly understood. **Objective.** This study to answer whether 4-PBA can decrease apoptosis triggered by inflammatory reactions mediated by TNF α . **Methods:** This study is an exploratory study laboratory in vitro using cell culture Human Umbilical Vein Endothelial Cells (HUVEC) apoptotic count cells with the design of post test only control group consisting of three treatment groups with doses of 4-PBA 1 nM / mL, 2 nM / mL, and 3 nM / mL. **Results.** Administration 4-PBA in cultured HUVEC derived endothelial cells significantly decrease apoptosis at any dose of PBA but no dose dependently ($p < 0.05$). **Conclusion:** Based on the results that has been done, it can be concluded that 4-PBA can reduce levels of endothelial cells apoptosis which were exposed to proinflammatory cytokine TNF- α . Further research need to elucidated 4-PBA mechanism to inhibit endothelial apoptosis.

Keywords: Apoptosis; Endothelial cells; Inflammatory; Phenylbutyrate acid 4-PBA

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Introduction

TNF- α has been widely known as a potent pro-inflammatory cytokine involved in many degenerative disease. Produced by macrophage, TNF- α has many pivotal role to stimulate cellular apoptotic initiation in inflammation reaction. Endothelial cells is one of its primary target organ-damaged which will lead to its dysfunction and undergone its cellular apoptotic¹. 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 of the blood vessel². Good quality of the blood vessel wall is determined by the quality of endothelial cell. Metabolic perturbation in endothelial cells will lead its dysfunction called endothelial dysfunction (ED). Chronic inflammation was one of the cause of ED and it has many manifestation³. Among of its molecular pathway is involvement of the endoplasmic reticulum (ER) organelles disturbance which is called the endoplasmic reticulum stress (ERS)⁴. ERS has been

grown as new areas of research that involve in many disease⁵. Endoplasmic Reticulum (ER) has its role in homeostasis regulation in intracellular calcium ion concentration, in the process of apoptosis, sterol biosynthesis and the release of arachidonic acid, ER has a main role in maturation of protein synthesis. Maturation of protein folding and post-translational modifications are placed in its lumen before secreted into the extracellular matrix, membrane structure, membrane lysosome and Golgi apparatus^{6,7}. In the process of protein synthesis, ER responsible for the folding process of proteins produced by the of attached ribosomes in outer layer of ER. ER lumen very sensitive in protein formation changes that disrupt its functionality. After going through the process of cotranslational translocation, nascent protein enter the ER lumen which is rich in oxidative environment. Nascent protein begin to folded in its hydrogen and sulfidic bond to get a more stable conformation and low energy. Protein folding in the ER lumen is unique compared to other cellular compartments. In luminal ER, support system of covalent folding

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machine is more complex consisting of dumping signal sequence, disulfide bond formation, N group glycosylation and glikosilfosfatidilinositol(GPI) group addition⁸. High protein demand and low capacity of ER lead to accumulation of a unfolded protein that is not folded properly lead to RE inability to overcome the high protein demand known as ER stress and elicit a response in the form of the unfolded protein response (UPR)[9]. As a result, the nascent protein produced by ribosomes will flood the ER lumen. Homeostatic mechanism increased ER capacity by increasing the amount of protein folding chaperones Glucose Related Protein 78(GRP78). If this mechanism is uncompensated, then the unfolded protein accumulates in the ER.ER chaperon GRP78 protein bound to the transmembrane proteins were then released from the ER membrane and activate UPR cascade[10]. There are three transmembran protein act as UPR molecular sensor IRE1, PERK dan ATF6. These protein will release from ER membrane when luminal GRP78 release from the other side of transmembran protein.

4-Phenylbutyrate acid (4-PBA) is a short chain fatty acid with a low molecular weight which has long been used in the treatment of thalassemia, sickle cell anemia and urea cycle disorders in children. His ability to capture ammonia is a feature that is used to treat urea cycle disorders in children while increasing the ability transkrips factor α and γ -globulin is a base in the treatment of thalassemia and sickle cell anemia¹¹. It is a classical prototype endoplasmic reticulum stress inhibitor acting as histondeacetylase inhibitor that lead to increase of folding protein HSP70¹². The use of 4-PBA as chemical chaperones began to emerge when PBA can reverse misfolding protein in its maturation that occurs in some pathological processes¹³. Further studies proved that 4-PBA had this ability in ER when protein load increases¹⁴. In drosophila melanogaster fly, 4-PBA administration prolong longevity¹⁵.

However, whether endoplasmic reticulum stressmechanism are involved in TNF-alfa induced endothelial apoptosis is uncertain and remains unexplored. In this paper, we investigated the effect of 4-PBA, an endoplasmic reticulum stress inhibitor, on TNF- α -induced apoptotic endothelial cells.

Materials and methods

Samples were umbilical vein obtained from umbilical cords of babyborn, after obtaining informed consent from their parents. The cord cutted in aseptic methods 20 cm length and soaked in a solution cord in sealed bottles. After sterilized with 70% ethanol,

each tip of the cord cutted transversely to exposed its arteries and vein. Vein had thicker walls, larger and elastic than arteries. A cannule inserted at one tip of the vein (\pm 1 cm), then tied tightly with string. Vein cleaned with 10 ml PBS with a 20 cc syringe through this cannula. Once it is clean, tie the other end of the umbilicus with strong ties or clamped. Collagenase solution then injected into its vein lumen. Umbilicus being warmed by cuddled methods with both hands or brought closer to the litle flame in order to reach a temperature of 37 ° C for 7 min. Collagenase which already contains endothelial cells then removed from the umbilicus through a syringe which was mounted on the tip of the cannula. Collagenase solution is placed in a sterile 15 cc centrifuge tubes. Umbilicus rinsed once or twice with 8 cc PBS solution to flush e endothelial cells remaining and added to centrifuge tubes containing a solution before. Solution which were already contains the endothelial cells were centrifuged at 1000 rpm for 8 minutes. The supernatant was discarded, then added 4 cc of the culture medium into pellet and resuspended in a way so that endothelial cells separate. The solution then transferred into a 0.2% gelatin coate 24 well plate then put in a 5% CO₂ incubator at 37 ° C for 20 minutes. Culture plate take and observed with inverted microscope magnification of 400x if the cell is attached to the base well. Cell culture rinsed with 3cc serum free medium and filtrate. Serum free medium then taken with a sterile syringe and replaced with culture medium 4 cc through a 0.2 mL filter. Culture plate put in an incubator until the monolayer (cobblestone form) approximately 3-4 days and the medium was replaced every 2 days. HUVECs in a 24-well plate then exposed with TNF- α 5ng/L and PBA at multiple dose after achieving optimal conditions¹⁶. HUVECs were fixed with methanol in glass slides, then rinsed with phosphate buffer solution (PBS). Normal human serum as blocking agent (1:10 dilution) (MPBio, USA) was applied and incubated for 30 minutes at 37°C. Hematoxylin eosin staining was performed. The slides were then covered with cover slips. Data was analyzed by one-way ANOVA and the difference between groups was analyzed by post hoc LSD comparison test. Data are expressed as mean \pm standard error of the mean (SEM). p- Values less than 0.05 were considered statistically significant.

Ethical clearance: The study was issue from Human Ethical Committee of Faculty of Medicine, Brawijaya University, Malang(No 141/EC/KEPK/S3/05/2016).

Results and discussion

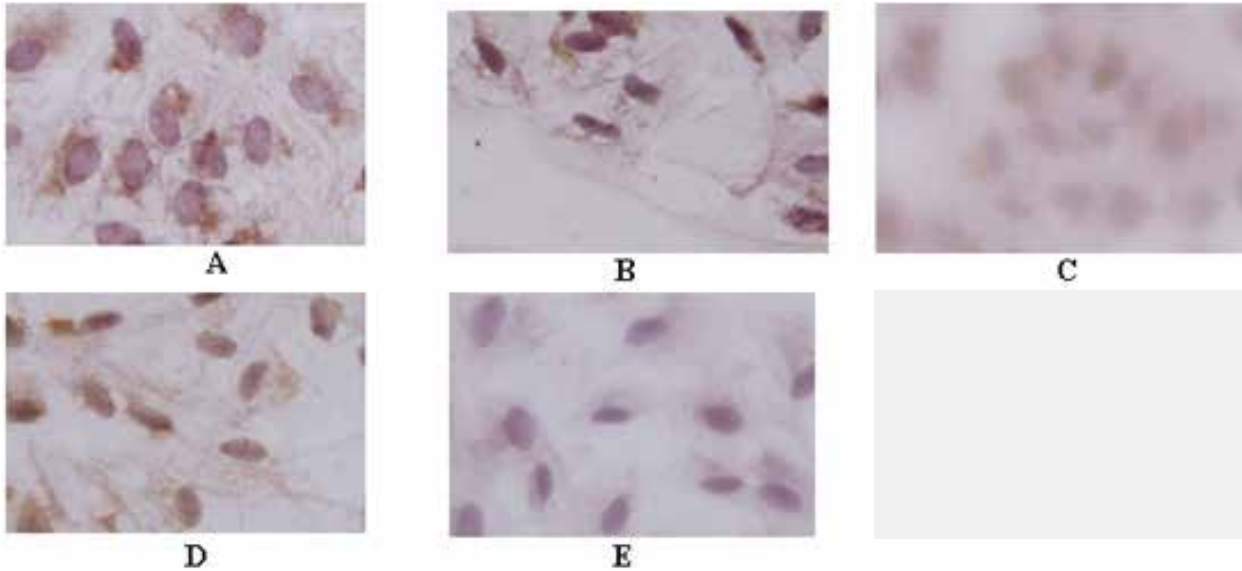


Figure 1. Endothelial cells TNF α -free control grup (A) has a minimum apoptosis (arrow) compare to TNF- α positive control (B). InPBA treat at dose 1 nM/L (C), TNF α + PBA2 nM/L (D) and TNF α + PBA3 nM/L (E) decrease its apoptosis. (magnification 400X)

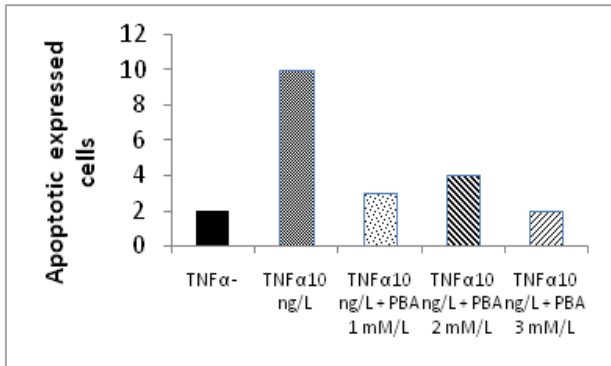


Table 1. Endothelial cells TNF α -free control grup (A) and TNF α positive control (B). InPBA treat at dose 1 nM/L (C), TNF α + PBA2 nM/L (D) and TNF α + PBA3 nM/L (E)

In this research TNF- α are significantly increase apoptotic cells in cultured human vein endothelial cells (B) compare with negative control (A). Administration of PBA significantly decrease apoptotic cells compare with positive control at any dose of PBA but no dose response relationship (CDE). This result consistent with other report but in different tissue such as in rat pancreas tissue¹⁷. PBA known have its ability as histone deacetylase inhibitor. Exposing endothelial cell to TNF- α will lead stimulation of NADPH oxidase 4 (NOX4) in cells. This stimulation will increase intracellular oxidative state and stimulate endoplasmic reticulum

stress and induce apoptosis. Inhibition ERS by PBA might have decrease its oxidative domino effect in ER through capasitation of protein folding in ER lumen.

Conclusion

Base from the research that has been done, it can be concluded that administration of PBA can reduce levels of piknotic cells in cultured TNF- α exposed HUVEC cells and at any dose of PBA at 1, 2 and 3 nM /L. More research is needed to answer the question whether PBA can reduce the expression of markers of endoplasmic reticulum stress else like GRP78, ATF6, Perk, IRE1. Therefore, further research is needed to elucidate the effect of PBA in reducing other markers of endoplasmic reticulum stress such as ATF6, IRE1 in different dose or in different interval.

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Conflict of interest

We declare none of conflict of interest in this research

Author Contribution:

Data gathering and idea owner of this study, study design, data gathering, writing and submitting manuscript & editing and approval of final draft all have done by Oski Illiandri

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