Abstract

The prevalence of hypertension is significantly higher in African Americans in comparison to other populations. Hypertension is a condition in which there is increased blood pressure in the arterial walls, which can lead to serious health problems such as heart attack and stroke. The African American population has been shown to have a higher risk of developing kidney failure than Caucasians due to hypertension. Apolipoprotein (ApoL) encodes the apolipoprotein L-1 protein (ApoL1) and has been shown to be prevalent in the African American population. Apocynin is a known inhibitor of NADPH oxidase (NOX), which catalyzes the reduction of molecular oxygen to the superoxide and has been shown to prevent the formation of reactive oxygen species, which decreases arterial stiffness in deoxycorticosterone acetate-salt-induced hypertensive (DSH) rats. A reduction in the amount of reaction oxygen species leads to a decrease in apoptosis. We hypothesize that because apocynin causes a reduction in reactive oxygen species, then it will cause inhibition of the PI3K/Akt pathway. In order to test this hypothesis, twenty-four male DSH rats were treated with a low salt diet (0.3% sodium chloride) with apocynin and aldosterone or a high salt diet (8% sodium chloride). The kidneys of the rats were homogenized and tested for the presence of APOL-1 using ELISA and Western Blot. The expression of NOX4 (an NADPH oxidase subunit) was also tested using ELISA. The rats that were fed a low salt diet exhibited the APOL-1 protein. In the presence of high salt or aldosterone, ApoL1 expression was completely inhibited. The expression of NOX4 was also seen in all of the rats (n = 24), but was higher in the rats fed a low salt diet (n = 12). Apocynin did not affect NOX4 expression, but did increase the production of reactive oxygen species. ApoL-1, along with apocynin, have an effect on Akt-signaling. However, instead of reducing the amount of reactive oxygen species, apocynin appears to be inhibited in the presence of the ApoL-1 protein and upregulates the Akt pathway. Future tests will involve identifying receptors in the Akt pathway that are affected by apocynin, which could lead to conclusions about the effect of apocynin and oxidative stress on the kidney.

Introduction

Disparities in the Prevalence of Hypertension in the African-American Community

The prevalence of hypertension is significantly higher in African Americans (AA) when compared with other populations. Hypertension is a condition that causes increased blood pressure in the arterial walls, which can lead to serious health problems such as heart attack and stroke (Kučera et al., 2015). If left untreated, hypertension can lead to chronic kidney disease (CKD). CKD is a slow occurring disease in which the kidneys lose function over a sustained period of time. The final stage of CKD is end-stage kidney disease (ESKD), which is the complete loss of function in the kidneys (U.S. National Library of Medicine, 2013). This is a permanent condition in which the kidneys are unable to filter waste and excrete excess fluid (Kao et al., 2008). The African American population has been shown to have a higher risk of developing ESKD from CKD than Caucasians (Petrônio et al., 2013).

The Role of APOL-1 in Hypertension in African Americans

Recent studies have shown that the abundance of hypertension in African Americans can be caused by several factors. Lower socioeconomic status, lack of access to healthcare, and higher systolic blood pressure are factors that lead to renal failure in the African American population. Myosin heavy chain 9 non-muscle (MYH9) is a gene that codes instructions for the synthesis of myosin-9. This gene aids in cell movement, cell shape, and cytokinesis. Previous studies using mapping techniques identified a peak on chromosome 22q13, which includes MYH9, that causes an increased risk of developing EKSD in African Americans. For many years, MYH9 was thought to have been the cause of hypertension in AA individuals. However, recent studies have shown that the gene responsible for this missense mutation is located near the MYH9 gene and is actually the apolipoprotein (APOL). APOL encodes the apolipoprotein L-1 protein (APOL1), which lyses trypanosome and is prevalent in autophagic pathways. More importantly, it has been shown that African Americans who have roots in the western parts of Africa have the apolipoprotein L1 gene. This gene is...
prominent in West Africa due to the occurrence of human sleeping disease, Trypanosomiasis, in that area. Human sleeping disease is caused by a parasite of the species Trypanosoma that is lysed by APOL-1. As a result, APOL-1 plays a significant role in the reduction of the human sleeping disease. However, African Americans living outside of Africa do not have the same exposure to human sleeping disease but still carry the gene for APOL-1. Thus, understanding the mechanism of action of APOL-1 can help lead to preventative methods against renal disease.

Research Question and Hypothesis

The PI3K/Akt signaling pathway is a critical pathway in the regulation of metabolism, growth, proliferation, survival, transcription, and protein synthesis. Akt is a mediator of cell survival through direct inhibition of pro-apoptotic proteins. Disruptions in this pathway is implicated in several diseases, including cardiovascular disease. Cardiovascular disease is the number one cause of death associated with high blood pressure. We hypothesize that because apocynin causes a reduction in reactive oxygen species, then it will cause inhibition of the PI3K/Akt pathway. The presence of ROS activates the PI3K/Akt signaling pathway. Therefore, we believe that the reduction in ROS caused by apocynin may interfere with the functioning of the PI3K/Akt pathway. By understanding the mechanism of action of apocynin, we can determine how a reduction in oxidative stress can affect hypertensive individuals. These findings can lead to conclusions about how reactive oxygen species can cause an increase in hypertension and a reduction in the number of phagocytes in the kidney. It has been proposed that a reduction in the number of phagocytes can occur, due to apoptosis of kidney cells caused by the prevalence of reactive oxygen species. This can lead to better understanding of the APOL1 gene and its mechanism of action in the kidney.

Materials and Methods

To test the hypothesis, we examined the effect of dietary salt (environment) and inhibition of ROS on ApoL1 expression in Dahl salt-sensitive (S) rats. The Dahl S rats were fed a low salt (0.3% NaCl) or a high salt (8% NaCl) diet alone or in the presence of apocynin, an NADPH oxidase subunit, for four weeks. Additionally, the low salt group was fed aldosterone to assess reabsorption of sodium. The amount of protein in the rats' urine was measured using protein assays at a 1:1000 dilution. Western Blots were then conducted to determine if the APOL-1 gene and NOX4, an NADPH oxidase subunit, were present in the rat. An ELISA was done to assess the oxidative stress.

Results

Figures 2 and 3 indicate the low salt group expressed both the ApoL1 protein and NOX-4. Low concentrations of salt could induce upregulation of the protein due to salt sensitivity that is prevalent in African America. Figures 2 and 3 indicate that aldosterone may have caused reabsorption of sodium in the kidney. Therefore, less salt would be excreted and more would be retained in the kidney. Additionally, these figures also indicate that the presence of apocynin does significantly decrease the presence of Apol-1. Lastly, the rats fed a high salt diet experienced the same results as the rats fed a low salt diet with aldosterone. This may be due to homeostatic regulation via antidiuretic hormone, which increases water reabsorption and increases sodium excretion.

Figure 4 is a combination of an ELISA performed using VEGF, TNF-alpha, IL-6, and MCP-1. These cytokines induce Akt-mediated signaling. Though none of the results were significant, the increase in the cytokines in the rats fed a low salt diet combined with Idosterone and apocynin indicate that the functioning of apocynin may be inhibited in the presence of the Apol-1 protein. Instead of decreasing the amount of ROS, they may accumulate in the hepatocyte, which is indicated by the increased cytokine activity of this particular group.
Figure 2. The above plots show the Western blot analysis for ApoL1 and NOX4.
Figure 3. Analysis of ApoL1 expression using ELISA. *LS/ALDO/APC (*) represent p<0.05 when compared to Low Salt group.

Figure 4a
Figure 4b

MCP-1

Figure 4c

TNF-Alpha
Figure 4abcd. ELISA results using VEGF, IL-6, MCP-1, and TNF-alpha. The aforementioned are cytokines that are involved in cell signaling in the immune pathway. These cytokines induce the PI3K/Akt signaling pathway.
Discussion and Conclusion

Using the rat ApoL1 ELISA, ApoL1 protein was only detectable in the rats fed a low salt diet. This can be explained by the high sensitivity of African-Americans to low concentrations of salt. In the presence of high salt or aldosterone, ApoL1 expression was completely inhibited in the kidney. Aldosterone tended to inhibit ApoL1 expression, but this inhibition was partially blocked in the presence of apocynin. The expression of NOX4 (an NADPH oxidase subunit) was also higher in the rats fed a low salt diet. Apocynin increased the production of reactive oxygen species. ApoL1, along with apocynin, have an effect on Akt-signaling. However, instead of reducing the amount of reactive oxygen species, apocynin appears to be inhibited in the presence of the ApoL1 protein and upregulates the Akt pathway. Future tests will involve identifying receptors in the Akt pathway that are affected by apocynin, which could lead to conclusions about the effect of apocynin and oxidative stress on the kidney.

References


H. Murata, Y. Ihara, H. Nakamura, J. Yodoi, K. Sumikawa, and T. Kondo, “Glutaredoxin exerts an antiapoptotic effect by regulating the redox state of...