The Effects of Chronic Nicotine Inhalation through Vaping on the Anticoagulant, Protein S, a Marker of Endothelial Cell Dysfunction, in Mice

Camden Hyde[1], Manoj Kumar PhD[1], Joshua Vedettal[2], Parijat Basak[1], Jason Gardner PhD[2], & Rinku Majumder PhD[1]

Department of Biochemistry[1], Department of Physiology[2], Louisiana State University Health Sciences Center, New Orleans, Louisiana

Introduction

Blood clotting, or coagulation, is an important process that prevents excessive bleeding when a blood vessel is injured. Platelets and proteins in plasma work together to stop the bleeding by forming a clot over the injury. Protein S (PS) is an essential natural anticoagulant and is present in the plasma in free and bound forms. PS deficiency is a major contributor to hereditary or acquired hypercoagulability. Hypercoagulability causes myocardial infarction, stroke, and deep vein thrombosis in millions of individuals. Protein S is associated with endothelial cell dysfunction. Decrease in Protein S and increase in thrombin generation suggest endothelial dysfunction. Chronic nicotine inhalation is also associated with the hardening of arteries, which can lead to cardiovascular disease, heart disease, and conceivably heart attack. According to 2020 statistics, 12.5% of the United States population were confirmed as nicotine users. In addition, 15% of global deaths are attributed to nicotine-induced heart disease. It is also known that cigarette smoking and nicotine use causes a prothrombotic state for its users.

It has been reported that Protein S is significantly downregulated in cigarette smokers. These alterations in Protein S may contribute to the thrombotic complications associated with smoking (1). In this study, we aim to determine whether chronic nicotine inhalation via vaping influenced thrombin generation and overall Protein S concentration in mice.

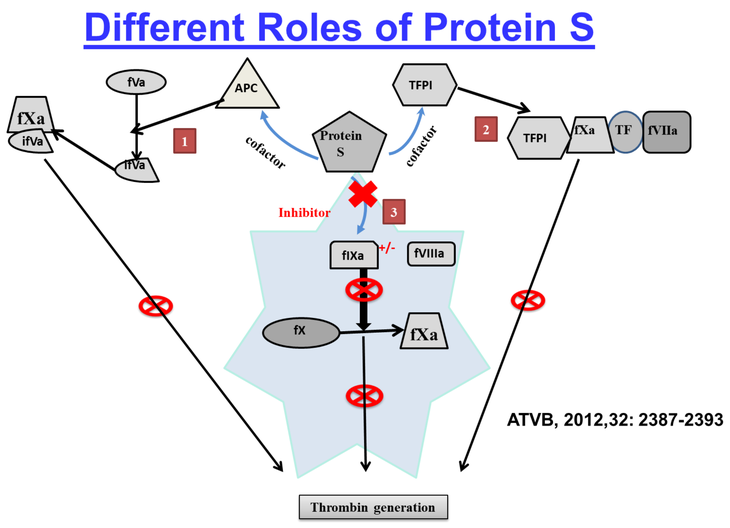

Figure 1. Coagulation cascade describing how protein S acts as a cofactor for activated protein C (APC), cofactor for tissue factor pathway inhibitor (TFPI), and aids in the down regulation of thrombin generation.

Experimental Design

- Mice were housed in a vape exposure chamber, or standard cages (air control). While housed in vapor chambers, mice were exposed to a vaporized liquid containing vegetable glycerin/propylene glycol (VGPG) and nicotine (Nic Salt). This vape liquid is used to mimic what is used in vaporized e-cigarettes, such as the JUUL. In addition, a mixture of VGPG ranging from 50/50 to 70/30 mixture with and without nicotine doses of 3% (low) and 5% (high) are used. These nicotine doses were selected based on what is commercially available in the JUUL and other e-cigarettes.
- Citrated plasma samples from Air, VGPG, and Nic Salt mice were given to us by Dr. Gardner's lab.
- We performed a western blot and probed for protein S. We also performed a thrombin generation assay (TGA) to allow us to monitor thrombin generation during coagulation.

Results

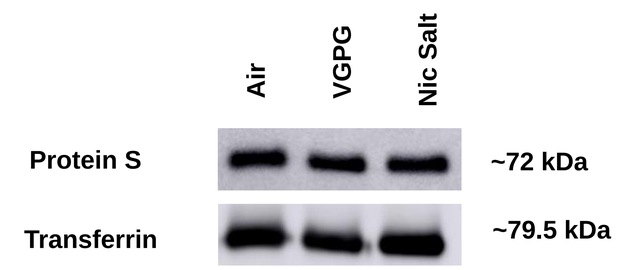

Figure 2. Western Blots of protein S from plasma isolated from air control mice (Air), vaping control mice (VGPG), and nicotine vaping mice (Nic Salt). Transferrin is loading control.

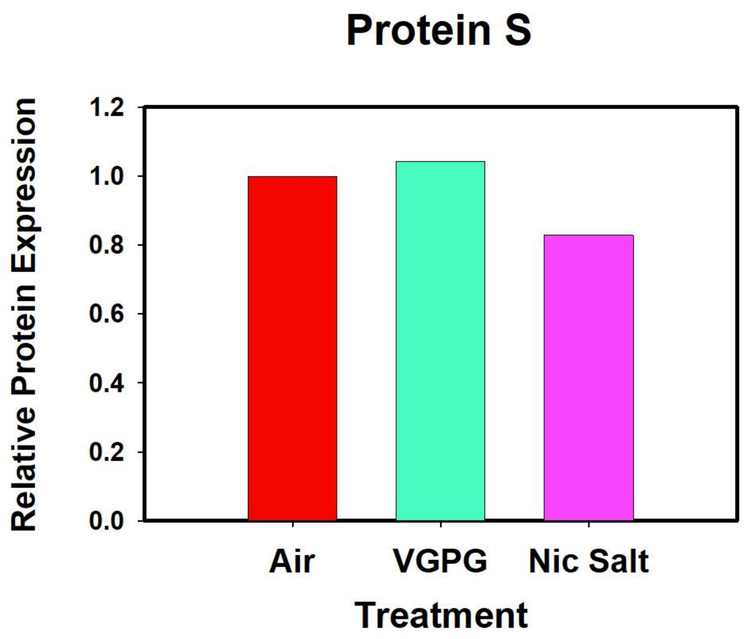

Figure 3. Comparison of relative protein S expression within air control mice (Air), vaping control mice (VGPG), and nicotine vaping mice (Nic Salt). This is based on western blot data. (keep same color for every figure treatment).

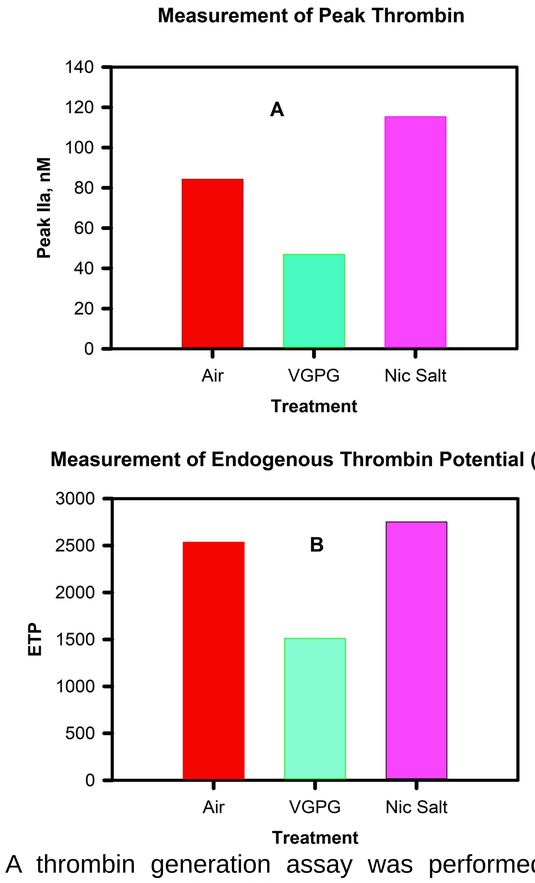

Figure 4. A thrombin generation assay was performed after isolating plasma from air control mice (Air), vaping control mice (VGPG), and nicotine vaping mice (Nic Salt). Graph A of Fig. 4 shows peak thrombin (IIa) generation and graph B shows endogenous thrombin potential (ETP).

Conclusion

- Our results show that protein S level decreases in nicotine vaping mice compared to mice that received the vaping control and air control.
- The thrombin generation assays showed that the peak thrombin (IIa) is highest among the nicotine vaping mice.
- Endogenous thrombin potential (ETP) is highest among the mice that received the nicotine vaping treatment.

Future Steps

- Increase the treatment weeks of mice used in this study.
- Investigate whether other endothelial cell markers like thrombomodulin and PAI-1 are affected with chronic nicotine inhalation.
- Perform an enzyme-linked immunosorbent assay (ELISA) to measure free protein S in mice plasma samples.

References

1) B D Scott 1, C T Esmon, P C Comp. The natural anticoagulant protein S is decreased in male smokers. Am Heart J., 1991,122:76-80.

2) Chattopadhyay, Rima., Sengupta,Tanusree., and Majumder, Rinku. Inhibition of Intrinsic Xase by Protein S. Arteriosclerosis, Thrombosis, and Vascular Biology, 2012, 32:2387-2393.

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