A PILOT STUDY: THE ACUTE EFFECT OF CAFFEINE ON MICE

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INTRODUCTION

Preeclampsia is a pregnancy specific syndrome, more specifically classified as a multisystemic, multifactorial vascular disease of pregnancy. Clinically, the syndrome is diagnosed by elevated blood pressure and proteinuria. Preeclampsia is the leading cause of maternal and fetal mortality, occurring in 3-5% of pregnancies [1].

Our lab believes that a possible link to preeclampsia is through nitric oxide synthase (NOS) pathway. Arginine, an amino acid, is covered to nitric oxide through the NOS. Nitric oxide increases dilation of blood vessels, angiogenesis, and trophoblast migration and invasion. Asymmetric dimethylarginine (ADMA) acts as an endogenous inhibitor to NOS. Previous studies have noticed that ADMA is elevated prior to the onset of preeclampsia and remains elevated postpartum [2]. Additionally, higher levels of ADMA have been shown to promote hypertension [3], impair endothelial-dependent vascular function [4], and impair angiogenesis [5].

The benefits of caffeine have also been considered in my lab, specifically the potential role it could have to treating preeclampsia. Literature has debated whether caffeine is beneficial or not. Several studies have claimed that caffeine is associated with an increased risk of hypertension [6]. While other studies have associated caffeine consumption to significantly decrease cardiovascular disease mortality in humans [7] and moderate caffeine consumption decreases the risk of preeclampsia [6]. A previous caffeine study explored the benefits of caffeine by administering a caffeine concentration of 2.5 mg mL⁻¹ [8]. Based on the uncertainty of caffeine and the unknown mechanism of preeclampsia, we devised a pilot study in mice.

OBJECTIVE

The pilot study objective is to treat mice with caffeine (2.5 mg mL⁻¹) over a short duration (1 week). The differences between the control and caffeine treated mice will be investigated by measuring the blood pressure for two weeks, measure the endothelial dependent vascular function, and the concentration of ADMA.

HYPOTHESIS/SUCCESS CRITERIA

Acute caffeine treatment will decrease blood pressure, improve endothelial-dependent vascular function, and decrease ADMA concentration in mice.

METHOD

The pilot study consisted of 10 male mice (between 2-3 months old). Mice were separated into two groups, 5 control mice and 5 caffeine treated mice. Mouse blood pressure was measured using a Kent Scientific Non-Invasive Blood Pressure System. Measurements were taken daily for two consecutive weeks. During the second week, caffeine concentration of 2.5 mg mL⁻¹ was administered to the caffeine treated group in the drinking water.

Mice were euthanized based on an approved protocol and mesentery and whole blood were collected. Endothelial-dependent vascular function was tested by a wire myograph system. Under a dissection microscope, mesentery arteries were dissected and cleaned from fat and veins. Each artery was strung with two tungsten wires and positioned within the jaws of a chamber unit. The vessels were contracted with a smooth muscle constrictor, phenylephrine (PE). Then, a relaxer, methylcholine, was added to the chamber unit by a serial dilution from 10 nmol L⁻¹ to 10 µmol L⁻¹ methylcholine. Force was measured and percent relaxation was calculated for each artery.

Mouse plasma was separated from the whole blood through centrifugation. ADMA was extracted from the plasma through an established detailed protocol, utilizing Micropore filters, and acidic and basic solutions. The filtered mouse samples were run through Waters Acquity Ultra Pressure Liquid Chromatography System. The ADMA concentration was measured in units of µmol L⁻¹.

The data was collected, and the mean and standard deviation were calculated for each experiment. Using a Student’s t-test, the control mouse data was compared to the caffeine mouse data. The data was considered statistically significant if the p value was less than 0.05.

RESULTS

During the first week without the caffeine treatment, the control group (n=5) had a systolic blood pressure 131±18 mmHg and diastolic blood pressure 103±17 mmHg. Similarly, the caffeine group (n=5) had a systolic blood pressure 132±21 mmHg and diastolic blood pressure 100±21 mmHg. After a week of caffeine treatment, the control group had a systolic blood pressure of 134±13 mmHg and diastolic blood pressure of 104±12 mmHg, while the caffeine had a systolic blood pressure of 119±24 mmHg and a diastolic blood pressure of 88±20 mmHg. Caffeine significantly reduced blood pressure in comparison to the control group with a p<0.05.

Demonstrated in Figure 1, control and caffeine treated mice had similar percent relaxation of contraction. At a concentration of 1 µmol L⁻¹ and 10 µmol L⁻¹ methacholine significantly improved endothelial-dependent vascular relaxation in the caffeine mouse group with a p<0.05.
Figure 1. Mean percent relaxation of contraction (%) with standard error bars of control and caffeine treated mice mesentery arteries.

Figure 2 displays the difference in ADMA concentration of the control group and caffeine group. The control group had a 0.24±0.05 µmol L⁻¹ ADMA concentration while the caffeine group had a 0.08±0.04 µmol L⁻¹ ADMA concentration. Caffeine significantly reduced ADMA concentration in the caffeine mouse group with a p<0.001.

DISCUSSION

The results of this pilot study did support our hypothesis that caffeine treatment significantly lowered blood pressure, increased endothelial-dependent vascular function, and lowered ADMA concentration. However, our pilot study had limitations. Male mice were used rather than female mice, thus the mice were not preeclamptic nor could they model a pregnant mouse. The study was also done in mice, rather than humans, hence the results cannot be directly applied towards humans.

Future direction of my project includes analyzing frozen organs for additional biological markers that are involved in the NOS. Also, using a female mice model rather than a male model would provide a more realistic setup.

Clinically, the pilot study may provide further insight into the pathways of preeclampsia. Furthermore, the pathway may explain part of the epidemiological link of caffeine consumption to decrease the risk of preeclampsia.

CONCLUSION

The acute effects of caffeine on male mice provided significant results in lowering blood pressure, improving endothelial-dependent vascular function, and decreasing the concentration of ADMA. The study supports other similar research findings. However, the pilot study has limited applicability. Given that the data is significant, further experiments and studies should be conducted to investigate the mechanism of preeclampsia.

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REFERENCES