Modulation of PON2 and Proinflammatory Cytokine Genes in Rat Tissue Exposed to Combined oral Contraceptive Ethinylestradiol and Levonorgestrel

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ABSTRACT

Paraoxonase (PON2) was identified as a genetic risk factor for cardiovascular disease (CVD) and usage of oral contraceptive (OC) is associated with increased cervical cancer and cardiovascular risk. PON2 protect against atherosclerosis development at the cellular level and this phenomenon could be related to their antioxidative properties. Therefore, the aim of the present study was to investigate the effect of OC on the expression of PON2, pro-inflammatory cytokines interleukin one alpha (IL1α) and tumor necrosis factor alpha (TNFα) in the liver, kidney and brain of rats. Different dosage groups of eight female rats were treated with oral contraceptive (0.15mg levonorgestrel 0.03mg ethinylestradiol(A); 0.3mg levonorgestrel 0.06 mg ethinylestradiol (B) and 0.075 mg levonorgestrel 0.015 mg ethinylestradiol (C))/kg body-weight(bw)). Two groups of eight rats were included in the study for a control group (D) and ≤0.1% DMSO (drug vehicle) group (E), which were not subject to drug administration for 21days. The levels of expression of the gene were assessed using quantitative reverse polymerase chain reaction technique. Combined oral contraceptive treatment produced a significant increase($p<0.001$) in the level expression of renal IL1α and TNFα in all the groups compared to control in a dose-dependent manner but has no significant effect on PON2. Meanwhile, OC resulted in significantly ($p<0.0001$) reduced level of expression of hepatic IL1α with no significant effect on hepatic PON2 and TNFα level. In the brain, OC resulted in significantly ($p<0.0001$) reduced level of expression of TNFα in all dose groups and IL1α level at 0.015mg/bw. Although OC treatment did increase the expression of brain PON2 significantly ($p<0.05$) at the lowest dose. Therefore, pharmacological modulation of the expression of genes could constitute a useful approach for preventing atherosclerosis.

INTRODUCTION

Oral contraceptives when used regularly are a very effective means of birth control. However, some women terminate their use because of worrisome side effects such as acne, high blood pressure, and weight gain. According to WHO, oral contraceptives adversely affect carbohydrate and lipid metabolism, and thrombolysis, this may be why they have been associated with an increased risk of cardiovascular disease(W.H.O, 1995). Epidemiological studies indicate a role for paraoxonase (PON) in cardiovascular diseases(Afolabi et al., 2016)

MATERIALS AND METHODS

Animal Exposure Model
- Healthy adult female rats weighing between (120-200 g) were exposed to combined OC (ethinyl estradiol and levonorgesterel)
- At various doses (0, 0.015, 0.03, 0.06 mg/bw) over a period of 28-days.

Tissue Collection
- Rats wetre euthanized by rapid decapitation to avoid contamination.
- Liver, Kidney and Brain were isolated and stabilized in RNA water solution prior to storage at -80 °C

RNA Extraction
- Total RNA was extracted from RNA*later* stabilized tissue using Aidlab EASYspin Plus RNA extraction kit following manufacturer's protocols.
- Tissue was pulverized and 20 mg of it was homogenized using electronic tissue homogenizer

cDNA synthesis
- cDNA was generated from mRNA and reverse transcriptase using protocols recommended by the manufacturer.

Data analysis
- Relative gene expression quantification data was generated and analyzed using the R Software.
- The GraphPad Prism 5 software version 6 was used for the statistical analysis

RESULTS

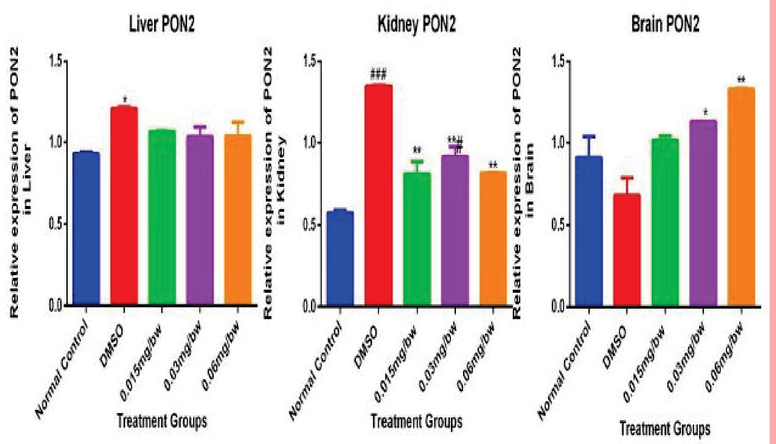

Fig. 1: Relative expression of PON2 in Liver, Kidney and Brain of rats exposed to different dosage of oral contraceptive. Each value is mean ± SEM for seven rats in each.

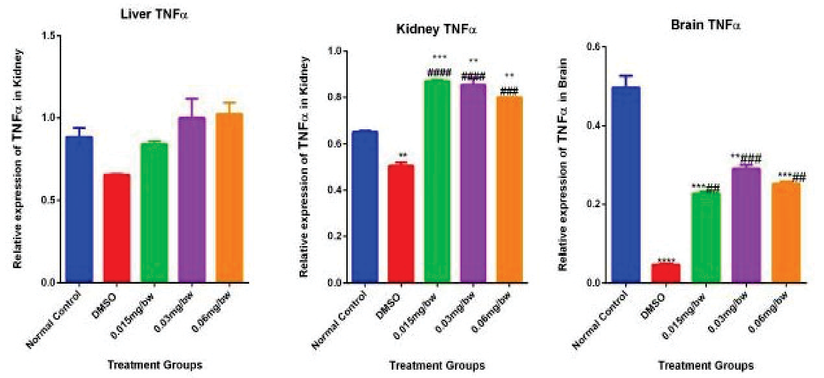

Fig. 2: Relative expression of TNFα in Liver, Kidney and Brain of rats exposed to different dosage of oral contraceptive. Each value is mean ± SEM for seven rats in each.

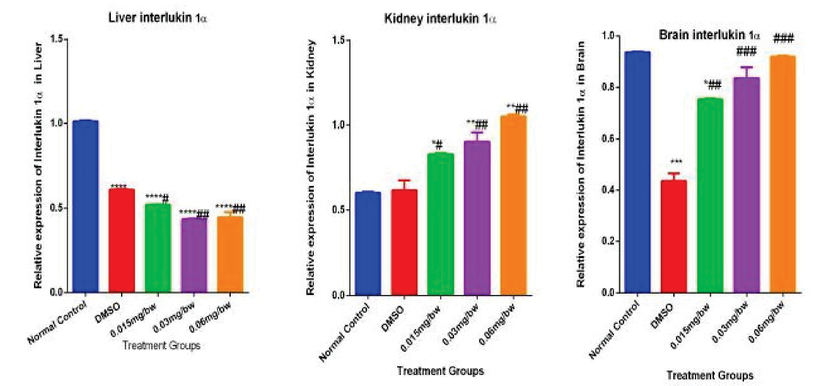

Fig. 3: Relative expression of Interlukin 1α in Liver, Kidney and Brain of rats exposed to different dosage of oral contraceptive. Each value is mean ± SEM for seven rats in each.

CONCLUSION

The finding of this study suggest that OC could predispose to cardiovascular risk. Although OC treatment did increase the expression of brain PON2 significantly at the lowest dose. Therefore, pharmacological modulation of the expression of genes could constitute a useful approach for preventing atherosclerosis.

REFERENCES

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