THE PROTECTIVE ROLES OF MICROGYNON AND NORDIOL IN CONTROLLING THE LEVELS OF LIPID PEROXIDATION: A COMPARATIVE STUDY ON FEMALE RATS

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ABSTRACT

Aims: This study aims to evaluate the effects of oral contraception (Microgynon and Nordiol) on the level of lipid peroxidation as a part of oxidative stress progression in female rats. Oral contraception were used. Materials & Methods: Thirty rats (females) were included in this study. They were divided into 5 groups (six in each group): a control group was maintained on normal standard chow diet, while the other 4 groups received high cholesterol diet for 14 weeks. After 14 weeks, blood samples were taken randomly from 6 rats. The remaining 18 rats were randomly divided into 3 groups (6 rats in each), the first group continued on high cholesterol diet and distilled water for 6 weeks and served as self-control group. The remaining 2 groups continued on high cholesterol diet and were given one of the oral contraceptives mentioned above for 6 weeks. Results: Our data showed that Microgynon treatment resulted in a significant reduction in serum GSH with no significant changes in MDA, CAT and GST activities. However, Nordiol treatment has no significant effects on serum level of GSH, MDA, CAT. Conclusion: The controversial findings proves the effects of the two types of the oral contraception on lipid peroxidation via different metabolic pathways.

KEYWORDS: Oral contraceptives, Lipid Peroxidation, Antioxidants.
INTRODUCTION

Oral contraceptives (OCs) contain synthetic forms of two hormones produced naturally in the body, the estrogen and progestin which regulate the female menstrual cycle.[1, 2] Some types of oral contraceptives use only progestational hormones, but most use a combination of estrogen and progestin. Combined oral contraceptives (COCs) prevent pregnancy primarily by inhibiting ovulation through the combined actions of progestin and estrogen.[1, 2] Although rare but serious potential side effects include hyperlipidemia, stroke, breast and liver tumors, and gallbladder disease.[3] Hypercholesterolemia is an important risk factor for the development and progression of atherosclerosis and premature coronary heart disease.[4, 5, 6] The pathogenesis is multifactorial and includes vascular inflammation and increased generation of vascular superoxide, hydroxyl radicals, and hydrogen peroxide.[7, 8, 9] These free radicals can cause oxidative stress.

Oxidative stress is defined as a state of imbalance between the rate of production of free radicals and the body's ability to scavenge them.[10] Continuous state of moderate to high levels of oxidative stress can leads to cell death (apoptosis). A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which are chemical species that have a single unpaired electron in an outer orbit.[11] They are highly reactive chemical radicals that are generated as products of oxygen degradation.[11] Oxidative stress plays an important role in the DNA damage, lipid peroxidation and contributes to the pathogenesis of atherosclerosis.[12]

Estrogen is known to be powerful antioxidant independently of their binding to the estrogen receptors and the hormonal functions.[13] Estrogen is widely regarded to have beneficial effects on lipid profile, these effects include a reduction in LDL-C, an increase in HDL-C, but it increases plasma triglyceride levels.[13, 14] However, progestins oppose the antioxidant effect of estrogen, with the strongest antiestrogenic effect seen with the synthetic progestins, levonorgestrel and medroxyprogesterone acetate.[14]

This study aims to clarify the effects of COCs in both high and low doses on serum oxidative stress parameters including serum reduced glutathione (GSH), serum malondialdehyde (MDA); two antioxidant enzymes catalase (CAT) and glutathione s-transferase (GST) and on serum lipid profile in female rats.
MATERIALS AND METHODS

Preparation of Animals

The experimental procedures and animal uses related to this study were approved by the Scientific and Ethical Committee of the College of Medicine, University of Kufa, according to the guidelines for the care and use of laboratory animals in scientific research. Thirty healthy adult female Sprague-Dawely rats were included in this study. Their weight range was between 170-250 g and aged between 3-6 months. The rats were housed in Kufa Medical College Animal House in 43 x 27 x 15cm cages (3 rats in each cage) and kept at 25 °C and 12 hours light-dark cycles with 12.00 AM being the mid-dark period. Rats had free access to drinking water and libitum. After 2 weeks of adaptation, 6 rats were maintained on normal standard chow diet and formed the control group from which the baseline values of experimental parameters were determined.

The other 24 rats received high cholesterol diet containing cholesterol 3% by weight made by the addition of cholesterol powder (Griffin Company, England) to the chow plus coconut oil 25% by weight,\textsuperscript{[15]} for 14 weeks to induce hypercholesterolemia. After 14 weeks, blood samples were taken randomly from 6 rats which underwent laparotomy and experimental parameters were measured. The remaining 18 rats were then randomly separated into 3 groups, six rats in each group as follows;

**Group 1:** Continued on high cholesterol diet and distilled water orally by gastric tube for 6 weeks without drug treatment and served as the control.

**Group 2:** Continued on high cholesterol diet and Microgynon (EE\textsubscript{2} 0.004906 mg/kg + LNG 0.024533 mg/kg) by stomach tube for 6 weeks.

**Group 3:** Continued on high cholesterol diet and Nordiol (EE\textsubscript{2} 0.008177 mg/kg + LNG 0.040889 mg/kg) by stomach tube for 6 weeks. At the end of the 6 weeks blood samples were taken from all rats which underwent laparotomy and experimental parameters were measured.

Sample Preparation

After an overnight fasting, 3 ml of blood sample was obtained directly from the heart of the anesthetized animal (with chloroform) which underwent laparotomy, without the use of heparin. The blood sampling was performed before and after 14 weeks of high cholesterol diet and after 6 weeks of drug treatment for each group.
Serum samples were prepared by centrifugation at 3000 xg for 10 minute to determine the experimental parameters which include GSH, MDA (the byproduct of lipid peroxidation), GST enzyme, CAT enzyme and lipid profile.

**Serum lipid profile assay**
Total cholesterol, Triglyceride and High density lipoprotein were measured according to procedures supplied by Spinreact company kits, using (Shimadzu UV-1650P (UV-visible)) Spectrophotometer. Serum LDL-C measure according to Friedewald equation.\(^{16}\)

\[
LDL-C = \text{total cholesterol} - \text{HDL-C} - \text{VLDL-C}
\]

\[
\text{VLDL-C} = \frac{\text{TG}}{5}.
\]

**Oxidative Stress/Antioxidants Parameters**
The amount of Serum lipid peroxidation product malondialdehyde (MDA) produced was measured using the thiobarbituric acid reacting substance method.\(^{17}\) MDA can react with thiobarbituric acid (TBA) to form a pink colored product which can be measured using spectrophotometer.

The levels of serum Glutathione (GSH), Serum glutathione s-transferase (GST) & Serum Catalase (CAT) enzymes were measured using EIA Assay Kits, Cayman Chemicals, USA.

**Dose of Contraceptive Hormones**
The dose of hormonal contraceptive pills in rodents per kilogram of animal weight has been measured as 10 times the human dose.\(^{18, 19}\) Considering the human dose is one tablet daily and the average weight of adult human female = 61.14 kg,\(^{18, 19}\) the dose is given once daily at the same time every day.

**Microgynon:** It was used in a dose of EE\(_2\) 0.004906mg/kg + LNG 0.024533mg/kg /day P.O, a tablet contains EE\(_2\) 0.03mg and LNG 0.15mg (microgynon ed fe [Schering]) was dissolved in distilled water and the dose was given to the rats according to the body weight once daily at the same time every day through stomach tube for 6 weeks.

**Nordiol:** It was used in a dose of EE\(_2\) 0.008177mg /kg + LNG 0.040889mg/kg /day P.O, a tablet contains EE\(_2\) 0.05mg and LNG 0.25mg (nordiol [Wyeth-pharma]) was dissolved in distilled water and the dose was given to the rats according to the body weight once daily at the same time every day through stomach tube.
Statistical analysis: Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) version 16.0 and Microsoft Excel (Office2007, Microsoft). The data were expressed as mean ± SEM unless otherwise stated. Analysis of Variance (ANOVA) was used for the multiple comparison among all groups. Statistical analyses between means were assessed by using independent t-test and least significant differences (LSD). Significant difference was set at α=0.05. Power analysis using a high power coefficient & effect size indicated that the number of samples included in the analysis were sufficient.

RESULTS

Effect of 14 weeks cholesterol rich diet on the measured parameters

Comparison between the rats on normal diet and on high lipid diet was as shown in table 1 where there were statistically significant increase in serum total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) in rats with high lipid diet compared to the rats on normal diet. There were no significant changes in serum GSH, CAT, MDA, and GST between both groups (Table 1).

Table 1: Oxidative stress parameters after 14 weeks of the study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Rats on normal diet N=6</th>
<th>Rats on high lipid diet N=6</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (µM)</td>
<td>29±3.68</td>
<td>25.5±2.32</td>
<td>N.S</td>
</tr>
<tr>
<td>CAT (k/ml)</td>
<td>0.05±0.008</td>
<td>0.04±0.002</td>
<td>N.S</td>
</tr>
<tr>
<td>MDA (M)</td>
<td>5.51*10^5±0.79</td>
<td>7.6*10^5±1.44</td>
<td>N.S</td>
</tr>
<tr>
<td>GST (U/L)</td>
<td>21.61±4.35</td>
<td>16.4±1.05</td>
<td>N.S</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>45.15±1.62</td>
<td>53.8±3.68</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>20.5±2.94</td>
<td>50.48±1.86</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>31.05±0.88</td>
<td>22.04±1.08</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>10.001±0.66</td>
<td>21.66±3.56</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>VLDL-C (mg/dL)</td>
<td>4.1±0.58</td>
<td>10.09±0.37</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

The values expressed as mean ± standard error of mean.

N.S: Non-significant correlation

Effects of the treatment on oxidative stress parameters

There were no changes of oxidative stress parameters in all groups except in microgynon group where there was a statistically significant decrease of serum GSH level after 6 weeks of treatment (Table 2).
Table 2: Levels of oxidative stress markers in response to contraceptive hormonal treatment

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>After Treatment</td>
<td>P value</td>
</tr>
<tr>
<td>GSH (µmol/L)</td>
<td>25.5±2.32</td>
<td>24.2±4.45</td>
<td>N.S</td>
</tr>
<tr>
<td>CAT (µmol/L)</td>
<td>0.04±0.002</td>
<td>0.04±0.003</td>
<td>N.S</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>7.60 x10⁻⁶ ±1.44</td>
<td>8.02 x10⁻⁶ ±0.83</td>
<td>N.S</td>
</tr>
<tr>
<td>GST (µmol/L)</td>
<td>16.4±1.05</td>
<td>15.93±1.48</td>
<td>N.S</td>
</tr>
</tbody>
</table>

The values expressed as mean ± standard error of mean

NS: non-significant correlation

Effects of the treatments on lipid profile

Serum lipid did not significantly change in the self-control group. Microgynon treatment decreased HDL-C level significantly but had no significant effect on serum TC, TG, LDL-C and VLDL-C levels. Nordiol treatment decreased serum HDL-C level, increased TG and VLDL-C levels significantly, but had no significant effect on serum TC and LDL-C levels. (Table 3).

Table 3. Levels of serum lipids in response to contraceptive hormonal treatment

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>After Treatment</td>
<td>P value</td>
</tr>
<tr>
<td>TC (mg/d)</td>
<td>53.8±3.68</td>
<td>54.38±2.56</td>
<td>N.S</td>
</tr>
<tr>
<td>TG (mg/d)</td>
<td>50.48±1.86</td>
<td>53.74±4.81</td>
<td>N.S</td>
</tr>
<tr>
<td>HDL-C (mg/d)</td>
<td>22.04±1.08</td>
<td>21.4±1.39</td>
<td>N.S</td>
</tr>
<tr>
<td>LDL-C (mg/d)</td>
<td>21.66±3.56</td>
<td>22.24±1.87</td>
<td>N.S</td>
</tr>
<tr>
<td>VLDL-C (mg/d)</td>
<td>10.09±0.37</td>
<td>10.94±1.01</td>
<td>N.S</td>
</tr>
</tbody>
</table>

The values expressed as mean ± standard error of mean

NS: non-significant correlation

DISCUSSION

In this study cholesterol rich diet didn’t induce a significant effect on serum GSH and MDA levels as well as CAT and GST enzymes activities (P>0.05). This can be explained by the fact that hypertriglyceridemia does not cause a significant oxidative damage[8,20] as hypercholesterolemia which is usually associated with more oxidative stress. In this study,
the cholesterol levels remained within the normal range (46-92 mg / dL),\textsuperscript{[21, 22]} hence there was no generation of enough reactive oxygen species that could potentially cause significant rise in the oxidative stress parameters.

Group 2 showed a statistically significant reduction in serum GSH level decreased significantly without a significant changes in the other parameters. The significant rise of the serum GSH in this group may be explained by the low level of estrogen content in Microgynon (which has an antioxidant effect).\textsuperscript{[23]} Other parameters of oxidative stress were not significantly changed in this study indicating that the presence of low level of estrogen may be helpful to reduce the accumulating free radicals without significantly changing the other parameters of the oxidative stress. However, other studies proved a significant reduction in the antioxidant enzymes activity.\textsuperscript{[24,25]} Massafra \textit{et al}, on the other hand, had found an increase in CAT activity during COCs treatment.\textsuperscript{[26]}

Group 3 had no significant changes in any of the parameters used in this study. This may be related to the high estrogen content in Nordiol that antagonizes the oxidative stress effect of levonorgestrel (LNG).\textsuperscript{[13]}

Serum HDL-C level decreased significantly while serum TC, TG, LDL-C and VLDL-C levels did not significantly change in group 2 (Microgynon treated female rats) as compared to group 1 (the self-control group). The molecular mechanism proposed was that LNG stimulated hepatic lipoprotein lipase which was involved in the degradation of HDL-C, when a combination of EE\textsubscript{2} 30 µg and LNG 150 µg was used. However, the effect of estrogen outweighed that of LNG on hepatic lipoprotein lipase resulting in a 30% increase. In spite of this, there was a significant reduction in the concentration of HDL-C.\textsuperscript{[27,28]}

Nordiol treatment group had a significantly increase in serum TG and VLDL-C levels associated with a statistical significant reduction in HDL-C level while TC & LDL-C did not significantly change. A similar molecular mechanism as in Microgynon was proposed for the decrease in HDL-C level since both contain the same ratio of EE\textsubscript{2}: LNG which is 1:5. The significant increase in TG may occur due to the estrogen component of the COCs, which increases serum TG level because of increased VLDL-C production.\textsuperscript{[13, 14]} In the present study serum, LDL-C level did not change.
The current study showed a slightly significant elevation in the degree of oxidative stress in association with Microgynon treatment. Such a change which couldn’t be proved with the use of Nordiol treatment. However, Nordiol treatment may causes more adverse changes in serum lipid compared to the first group.

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