Genotoxic Effects of Depo-Provera on Adult Female Rats

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Abstract: Rational: Depo-Provera©; (Medroxyprogesterone Acetate, DMPA) is a long-acting, injectable progesterone derivative contraceptive that is currently used by 30 million women in 90 countries, including the United States and Canada. Objective: DMPA classified as sex hormone binding globulin (SHBG). In this study the effect of (DMPA) on Deoxyribonucleic acid (DNA) was investigated on rats. Design: Sixty mature healthy female rats (Rattus norvigus) with average body weight ranged (130 – 200 gm) were injected intramuscularly with DMPA human therapeutic (150 and 300 mg) equivalent to rat doses (2.7 mg or 5.4 mg/ rat) and sacrificed after 10 and 15 days of the treatment. Methods: DNA was extracted from liver tissue samples using SS-phenol / chloroform, quantified by spectrophotometer and separated electrophoretically on agarose gel. Gels were photographed images were analyzed using RFLP software version Windows. The Student's “t”-distribution were adopted for assessment of significant changes occurring between the groups. Results: DMPA induced decrease in the concentration of the nucleic acid (DNA) as well as DNA damage manifested in fragmentation and adduct formation. Conclusion: The findings of this study shed more light and traceable to effects of this contraceptive agent on the adult female rats. It also suggests a genotoxic potential of DMPA and advisable to be careful of the potential hazards of DMPA. This demands the lowest possible use of effective and acceptable doses of these drugs so as to minimize any potential risk. Otherwise they may become capable of attacking the genetic material.

Key words: DMPA, female rats, Electrophoresis and DNA.

INTRODUCTION

Depot medroxyprogesterone acetate (DMPA) is a highly effective contraceptive method. It has been used as a contraceptive agent by millions of women in more than 90 countries since 1967 and was approved for use in several developing countries in 1992. In Egypt, the majority of the Egyptian women who are using contraception use DMPA as their birth control method. With the increasing use of female contraceptives by women for the purpose of family planning necessity was felt for this study to observe the effect of female contraceptives on the DNA. The risk of DNA damage in relation to hormonal steroid DMPA which have a widespread use in medicine as contraceptive and their side effects are continuously debated. The possible genotoxic activity of steroids has been the subject of many investigations. Progestins include the naturally occurring hormone progesterone and several synthetic compounds with progestational activity. Progesterone is rarely used therapeutically, whereas the synthetic progestins are more often employed either alone or combined with an estrogen for contraception, and in conjunction with estrogens for hormone replacement therapy in post-menopausal women. Progestins have been recently classified by the International Agency for Research on Cancer[12] as possibly carcinogenic to humans on the basis of sufficient or limited evidence for carcinogenicity in experimental animals and inadequate evidence for carcinogenicity in humans. Several authors reported on the effects of Progestins on the nucleic acids of the experimental animals, Korukhov and Kudriavtseva[15] examined the effect of contraceptive doses of Mestranol and Noiethinodrel on the nucleic acids in the uterus and liver in sexually mature female-rats which induced increase in the concentration. Cyproterone acetate (CPA) induced Hyperplasia of the rat liver by decreasing liver DNA content by about 25% within a few days of treatment[3]. Mitogenic, tumorigenic and induction of DNA-adducts and DNA-repair synthesis in rat liver increased at high Cyproterone acetate (CPA) doses[16]. Furthermore Lanari et al.[13] DMPA induces progesterin-dependent ductal metastatic mammary tumors with high levels of Estrogen receptor (ER) and Progesterone receptor (PR) in female BALB/c mice. DMPA
were converted from human dose to rat dose by using animals were injected intramuscularly with DMPA and the last two groups were treated groups. These animals were injected intramuscularly with DMPA doses at the first day of the experiment. The doses were converted from human dose to rat dose by using multiplication factors for dose conversion between different species by Paget and Barnes [22]. Nucleic acid (DNA) was extracted from liver tissue samples using SS-phenol / chloroform, determined by spectrophotometer and electrophoretically on agarose gel separated according to [14] and modified by [7]. DNA gels were photographed using Polaroid Camera supplemented with hoods of variable size and Polaroid films model (667). After photography the images were analyzed using RFLP software version Windows. The Student’s “t”-distribution were adopted for assessment of significant changes occurring between the groups.

RESULTS AND DISCUSSION

Liver DNA Concentration (mg/mL): The data obtained from treated animals at 10 and 15 days, with 150 and 300 mg equivalent doses of DMPA led to a dose-related decrease in the concentration of DNA (maximum ~ - 60.00 %). These decreases were statistically highly significant when compared with the control (Table 1).

Electrophoretic Assay: DMPA induced several discomfitures in the pattern of DNA bands separated on agarose, these discomfitures represented by fragmentation and smearing. The number of DNA bands isolated from treated rats at ten and 15 days ranged between (1 - 4) bands, which reflect the ability of DMPA to induce DNA damage (Table 2 and plate 1).

Referring to similarity based on band matching of DNA bands dendrogram was constructed to assess this relationship between the resulted bands of the control and treated groups. Dendrogram based on simple band matching revealed that, there is no similarity between control group and the resulted bands of all treated groups (GII, GIII, GV & GVI) (similarity index = 0.00) as shown in Fig. (2). While, similarity index between (GIII&GVI = 66.67 %), (GIII, GVI& GII = 42.86 %) and (GII, GIII, GVI & GV = 30.00 %).

Cluster Analysis of DNA:
Similarity based on Multigraph: Similarity index between control and treated groups profile using multigraph illustrated in Fig. (1). The profiles from treated groups (GII, GIII, GV & GVI) showing decrease in profile similarity to the control profile which ranged between (0.00 - 46.75 %), which reflect the effect of the DMPA on the DNA pattern.

Also, dendrogram based on band matching and amount of protein in bands revealed that there is no similarity between control group and the resulted bands of all treated groups (GII, GIII, GV & GVI) (similarity index = 0.00) as shown in figure (3). While, similarity index between GII, GIII, GVI & GV reached 9.14 %.

MATERIALS AND METHODS

The present study was carried using mature female rats (Rattus norvigus) of an average body weight (130 – 200 g). They were apparently normal, healthy animals. They were housed in animal houses under regular periods of dark and light i.e. (12-hrs dark and 12 -hrs light) at room temperature. Animals were fed on standard rodent pellet diet with water. These animals were divided into two major groups named 10th and 15th days. Each major group divided into three minor groups, the first was considered a control group and the last two groups were treated groups. These animals were injected intramuscularly with DMPA doses at the first day of the experiment. The doses were converted from human dose to rat dose by using...
Table 1: Effect of DMPA on the liver DNA Concentration (µg/µl) of the treated female rats in comparison with the control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>95% C.L.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>1.89 ± 0.03</td>
<td>1.75 – 2.00</td>
<td>1.83 – 1.96</td>
<td>100 %</td>
</tr>
<tr>
<td>GII</td>
<td>1.33’’ + 0.06</td>
<td>1.07 – 1.67</td>
<td>1.24 – 1.51</td>
<td>- 42.10 %</td>
</tr>
<tr>
<td>GIII</td>
<td>0.89’’’ + 0.03</td>
<td>0.78 – 1.12</td>
<td>0.88 – 1.01</td>
<td>- 52.91 %</td>
</tr>
<tr>
<td>GIV</td>
<td>1.60 ± 0.02</td>
<td>1.58 – 1.75</td>
<td>1.59 – 1.68</td>
<td>100 %</td>
</tr>
<tr>
<td>GV</td>
<td>1.00’’ + 0.03</td>
<td>0.83 – 1.16</td>
<td>0.93 – 1.06</td>
<td>- 60.00 %</td>
</tr>
<tr>
<td>GVI</td>
<td>0.73’’’ + 0.06</td>
<td>0.55 – 1.12</td>
<td>0.69 – 0.96</td>
<td>- 54.37 %</td>
</tr>
</tbody>
</table>

Where: SD = Standard Deviation. % = Percentage of change from control. ** = Highly Significant.
(’) = Decreased from control. 95% C.L. = 95% Confidence Limits.

Table 2: Molecular weights (bp) and Area percentage (A %) of separated genomic DNA from control and treated rat groups with DMPA doses for ten and 15 days.

<table>
<thead>
<tr>
<th>Bands</th>
<th>Ladder</th>
<th>Control</th>
<th>GII</th>
<th>GIII</th>
<th>GV</th>
<th>GVI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MW</td>
<td>MW</td>
<td>MW</td>
<td>MW</td>
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<tr>
<td>1</td>
<td>10000</td>
<td>9818</td>
<td>10091</td>
<td>10045</td>
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<tr>
<td>2</td>
<td>8000</td>
<td>--</td>
<td>7923</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>5000</td>
<td>--</td>
<td>5273</td>
<td>3758</td>
<td>--</td>
<td>3667</td>
</tr>
<tr>
<td>4</td>
<td>5000</td>
<td>--</td>
<td>5273</td>
<td>3758</td>
<td>--</td>
<td>3667</td>
</tr>
</tbody>
</table>

Where: MW = molecular weight in (bp), A % area percentage, (GII&GIII) treated groups for ten days. While, (GV&GVI) treated groups for 15 days.

Plate 1: Electropherograph pattern of genomic DNA from DMPA treated rat groups.
Where: A: Ladder; B: Control; C&D: Treated group GII&GIII for ten days. While E&F: Treated group GV&GVI for 15 days.
Fig. 1: Multigraph showing the relationship between DNA bands from both treated and control female rats at both ten and 15 days. Where: Lane 2 = Control, Lane 3 = GII, Lane 4 = GIII, Lane 5 = GV and Lane 6 = GVI.

Fig. 2: Dendrogram showing the relationship between DNA bands from both treated and control female rats at both ten and 15 days. Where: Lane 2 = Control, Lane 3 = GII, Lane 4 = GIII, Lane 5 = GV and Lane 6 = GVI

Fig. 3: Dendrogram showing the relationship between DNA bands from both treated and control female rats at both ten and 15 days. Where: Lane 2 = Control, Lane 3 = GII, Lane 4 = GIII, Lane 5 = GV and Lane 6 = GVI

Discussion: In this study DMPA was found to cause reduction in the concentration of deoxyribonucleic acid (DNA). While, the electrophoretic study of DNA showed that DMPA induced DNA damage which represented by fragmentation and adduct. It was suggested that the DMPA may be interacted with the nitrogenous bases of deoxyribonucleic acid (DNA) or phosphate groups at –OH site which will leads to formation of DNA complex structure (DNA adduct) which may leads to inhibition of DNA replication and decrease of amount of DNA as well as DNA fragmentation as clarified on agarose gel electrophoresis of DNA bands of the treated rats at both ten and 15 days of study. Similar results were reported by
The damage induced in liver DNA discussed by several authors and referred to various reasons as following: The steroid has been shown to induce DNA repair synthesis in rat and human hepatocytes and to form DNA adducts in rat liver cells. Formation of DNA adducts has also been shown in vivo in rat liver. In female rats DNA adducts have been observed at low doses of the steroid drug, which are in the range of the therapeutic doses used in women. DNA adduct formed due to binding of steroid hormone to DNA nitrogenous bases as adenine, guanine, thiamine and cytosine, as this takes place between liver DNA of rats treated by synthetic steroid cyproterone acetate (CPA) which was bound to guanine base. DNA adduct formation is considered to be a critical event in the multistage process of tumorigenesis. The progestogens CPA and DMPA or their metabolites were DNA reactive in male and female rat liver cells, since the involvement of the steroid molecule into DNA binding has only been demonstrated as reported by while trying to explain DNA damage occurred in rat liver cells treated with progestogens. Estrogen as steroid sex hormone and contraceptive when administered to rodents results in various types of DNA damage and ultimately leads to tumors in estrogen-responsive tissues. Also, the results of the present study in agreement and confirmed by the studies of genotoxicity which recently reviewed and DNA adduct formation or single strand breaks (SSB) as a marker for DNA breakage. Human hepatocytes in vitro were examined for UDS, SSB or 32P-postlabelling. All of these genotoxicity tests were positive and the responses by rat hepatocytes in vitro or in vivo were far stronger in females than in males. Since, the etiology of hormone-induced cancers has been considered to be a combination of genotoxic and epigenetic events. Braz and Salvadorei were studied the genotoxic potential of endogenous and synthetic sex hormones, as detected by the Comet assay. The data of the study indicated lack of genotoxicity induced by the physiological level of the female sex hormones and oral contraceptives as assessed by the alkaline Comet assay, which contradicted with the present results. However, the presence of DNA adducts have been reported in several genotoxic studies with many members of progestine family like Megestrol acetate, Cyproterone acetate and Chlormadinon acetate as published. Also, apoptosis was observed in human endometrial endothelial cells (HEECs) incubated with progesterone, levonorgestrel and medroxyprogesterone acetate. In conclusion this study concluded that DMPA is the most common injectable contraceptive, it was approved by FDA in 1992 and widely used world-wide by millions of women, which makes this an important issue that is worthy of animal trials studying possible ill-effects.

So, the genotoxic effects of this medication should attract the attention towards the risks of such medication on the women health. Also, the problem of long term synthetic hormone receiving (for contraception / hormone replacement therapy) is considered among the most serious ones that face mankind in this era. It is also supposed to be one of the greatest national problems, which will require special and intense effort at all levels individual, groups, national, and international. This is especially true regarding contraceptive medications (especially DMPA) which exert a high genotoxicity in the female rats. Thus, further epidemiological data on the long-term effects of DMPA injection on women should be warranted to the effect of DMPA on women health especially for the women in the Middle East region.

REFERENCES


