Effects of oxymetholone on kidney tissue of one-day old offspring of pregnant rats

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Abstract:

Introduction:
Oxymetholone is a drug used by some athletes as energy booster. However, its overuse may result in disorders such as lung cancer, ovarian cycle irregularities, liver cancer and adenoma of renal tubules. The aim of this study is to investigate the effects of oxymetholone on kidney tissue of one-day offspring of pregnant rats.

Material and Methods:
In this experimental study, 30 pregnant rats were divided into control, sham and experiment groups. The experiment group and the sham group were respectively administered 10 mg oxymetholone and 10 mg dimethyl sulfo oxide intraperitoneally for 15 days. On first day after delivery, the neonates were weighed and dissected. Kidney weight, length, width, and thickness were measured. Subsequently, tissue sections were studied microscopically. Data were analyzed using one-way analysis of variance with SPSS software.

Results:
The findings indicate that fetal weight, kidney weight, length, width and thickness, glomerular diameter, and cortical and medullary thickness in the experiment group were significantly greater compared to the control group (P<0.05), while the diameter of the interglomerular space was diminished. Moreover, certain pathological changes, such as glomerular cell proliferation, necrosis of cortical tubules and cast formation inside tubules, were observed.

Conclusion:
Oxymetholone, at the dosage used in our study, induces pathological changes in kidney tissue, as well as increased body and kidney weight in rat neonates.

Keywords: Oxymetholone, Kidney, Tissue

Introduction:
Oxymetholone is an anabolic drug synthesized by Ringold in 1959. It is a 17-alpha-alkamine and a synthetic derivative of testosterone, synthesized by methylation of 17-alpha carbon and saturation of 5-alpha carbon in testosterone. In addition, a hydroxymethyl group is placed in the position of carbon atom number 2 (1). Anabolic-androgenic drugs, particularly oxymetholone, are used at small doses (1-5 mg/Kg) for treating anemia, failure to thrive in children, and heart failure (2). These drugs increase erythropoietin production and
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probably affect bone marrow directly to increase hemoglobin and red blood cell (3). In addition, oxymetholone promotes nitrogen retention, protein synthesis and calcium deposition on bones (4). Furthermore, it increases sodium, potassium and chlorine salts, protecting athletes from injuries during intense exercises (5). Anabolic-androgenic steroids must be used at 10-100 times their normal (therapeutic) doses to assume anabolic properties, and their adverse reactions occur at these high doses (6). Prior to 1936, it was unknown that anabolic steroids boost muscle tissue. From that time on, anabolic steroids began to be used as performance enhanceers (7). Anabolic steroids were abused by Olympics weight lifters in 1954, and their abuse has gradually spread to other fields of sports (8). Nowadays, abusing anabolic-androgenic steroids is a hot issue throughout the world. According to reports issued by the National Olympics Committee, half of doping cases pertains to abusing anabolic agents (9).

Anabolic drugs, especially oxymetholone, are used by athletes for enhancing muscle growth and force. These substances delay cell death, but cause complications such as hepatic cancer, acne, premature alopecia, hirsutism in women, increased serum lipid (LDL) and cardiac diseases (9, 10). They may also result in behavioral complications such as aggression, anger, and violent behaviors (11, 12). In the present study, we investigate the tissue and pathological effects of oxymetholone on kidney tissue at higher than physiological dosages in one-day old rat neonates.

Material and Methods:
For the present study, we obtained 30 female and 10 male healthy adult Wistar rats from Razi Immunization Institute, Shiraz. Female rats were kept in trios in special cages and the male rats were kept in separate plastic cages in proximity of the cage of female rats in vivarium. The male rats were used for fertilization of female rats. Conception and pregnancy were confirmed with observation of vaginal plaque, after which the male and female rats were separated. The females were assigned to control, sham and experiment groups. During 15 days of pregnancy, rats in the experiment group were injected intraperitoneally with 10 mg/Kg/day oxymetholone, and rats in the sham group were injected intraperitoneally with 10 mg/Kg/day dimethyl sulfo oxide. Rats in the control group were preserved under normal conditions of diet and temperature. After delivery, the neonates were isolated from their mothers and weighed on scales. 5 neonates from each mother rat in the experiment group, and 3 neonates from each mother rat in the sham and control groups were anesthetized with ether and dissected. In order to extract the kidneys, the rat was fixed onto Styrofoam with pins, its abdomen was incised, and the kidneys were carefully removed using pincers and scissors with fine blades. The kidneys were then irrigated with physiologic serum, their dimensions were measured using a vernier caliper, and weighed using precise analytical scales. The specimens were then fixed, sectioned and stained with Hematoxylin-Eosin stain to be studied histologically with light microscopy. We used graticule and Dinolit software to measure cortex thickness, medulla thickness, glomerular diameter, and interglomerular space and assess them for pathologies. Subsequently, microscopic images were taken using a photomicroscope. Since Clark (1997) used 12 mg/Kg oxymetholone, which is almost 1 million times the normal dose of testosterone in rat’s body, to investigate ovarian cycle, we used a close dose of 10 mg/Kg in the present study. The resulting data were analyzed using one-way analysis of variance (ANOVA) with
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SPSS software. P values < 0.05 were considered significant.

**Results:**

Our findings indicate that in the experiment group, the weight of one-day old neonates, as well as kidney weight, length, width and thickness, cortical and medullary thickness, glomerular diameter, and diameter of the Bowman’s capsule space were significantly different from the control group (p<0.05). In other words, all these parameters, except for the diameter of Bowman’s capsule space, were significantly greater in the experiment group compared to controls. The diameter of Bowman’s capsule space was significantly smaller in the experiment group compared to controls (Tables 1, 2).

<table>
<thead>
<tr>
<th>Observation</th>
<th>Body Weight (g)</th>
<th>Kidney Weight (g)</th>
<th>Kidney Length (mm)</th>
<th>Kidney Width (mm)</th>
<th>Kidney Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>5.8800 ± 0.12497</td>
<td>0.0353 ± 0.00157</td>
<td>5.4867 ± 0.10111</td>
<td>3.300 ± 0.04819</td>
<td>2.3933 ± 0.010427</td>
</tr>
<tr>
<td>Sham Group</td>
<td>5.8867 ± 0.11868</td>
<td>0.0353 ± 0.00115</td>
<td>5.4867 ± 0.10134</td>
<td>3.2867 ± 0.04254</td>
<td>2.3733 ± 0.07013</td>
</tr>
<tr>
<td>Experiment Group</td>
<td>6.6423 ± 0.15347</td>
<td>0.041 ± 0.00182</td>
<td>5.9000 ± 0.12031</td>
<td>3.5525 ± 0.07604</td>
<td>2.8358 ± 0.07927</td>
</tr>
<tr>
<td><strong>P Value</strong></td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
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</table>

<table>
<thead>
<tr>
<th>Observation</th>
<th>Cortical Thickness (μ)</th>
<th>Medullary Thickness (μ)</th>
<th>Glomerular Diameter (μ)</th>
<th>Interglomerular Space Diameter (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>393.24 ± 11.8873</td>
<td>496.40 ± 15.0934</td>
<td>22.1300 ± 0.31690</td>
<td>4.6933 ± 0.13746</td>
</tr>
<tr>
<td>Sham Group</td>
<td>397.87 ± 14.9239</td>
<td>495.90 ± 11.6060</td>
<td>22.2333 ± 0.42765</td>
<td>4.7267 ± 0.11773</td>
</tr>
<tr>
<td>Experiment Group</td>
<td>465.10 ± 7.4565</td>
<td>535.76 ± 10.4765</td>
<td>23.4075 ± 0.37417</td>
<td>4.3475 ± 0.08251</td>
</tr>
<tr>
<td><strong>P Value</strong></td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</tbody>
</table>

On pathological investigations, we observe proliferation of glomerular cells, a lesion with homogenous involvement of glomeruli and enlargement, hyperplasia and a clover-leaf appearance of glomeruli. Moreover, it is obvious that glomeruli have lost their cluster form, mesangium is dispersed annularly outside the capillaries resulting in thickening of capillaries (Figure 1), necrosis of tubules in cortex, increased eosinophilia of tubule of tubular epithelial cells, tubular destruction (Figure 2), and cast formation inside tubules (Figure 3).

![Figure 1: Section of a one-day rat’s kidney from the experiment group (magnified x400; H&E staining)](image-url)
Figure 2: Section of a one-day rat’s kidney from the experiment group (magnified x400; H&E staining)

Figure 3: Section of a one-day rat’s kidney from the experiment group (magnified x400; H&E staining). Arrow marks the cast

Figure 4: Section of a one-day rat’s kidney from the experiment group (magnified x400; H&E staining)
**Conclusion:**
In this study, oxymetholone induced an increase in body weight, kidney weight, length, width and thickness, cortical and medullary thickness, and glomerular diameter in one-day rats, as well as a decrease in diameter of Bowman’s capsule space. Steroid hormones enter cells easily due to their lipid core and exert their effect by acting directly on nucleus. They promote DNA transcription and increase intracellular mRNA production, which results in increased synthesis of proteins necessary for the hormone’s response. This response promotes muscle and bone growth through synthesis of structural proteins, or modifies body physiology and function (13). Oxymetholone is an oral anabolic energy booster synthesized from 17-alpha alkylation of testosterone. Compared to testosterone, oxymetholone has high anabolic and low androgenic properties. Its anabolic effects are mediated through increased nitrogen retention and consequently increased protein synthesis (14, 15).

Nandrolone is another anabolic medication. It has been reported that nandrolone increases kidney weight and volume, cortical volume, and volume of the proximal and distal convoluted tubules in the experiment group compared to controls (16).

Considering the structural similarities of nandrolone and oxymetholone, the increase in kidney volume and weight, as observed in our study, may be attributed to oxymetholone effects. Androgens may induce renal disorders through altering the amounts of cytokinases and growth factors. It is believed that transforming growth factor plays a pivotal role in nephropathy by inducing the proliferation of mesangial cells (17). The proliferation of glomerular cells observed in our study is probably the result of oxymetholone effects.

A study by Deshmukh et al. reported that anabolic-androgenic steroids are excreted as glucoroid compounds which may create casts in the urinary tubules (18). This may account for the observation of cast in renal tubules in our study. Previous studies suggest that oxymetholone crosses placenta readily and affects the fetus (19). Thus, the variations and lesions observed in the experiment group of our study may be attributed to oxymetholone effects.

**Conclusion:** In general, it may be stated that at the dosage used in our study, oxymetholone induces pathological changes in kidney tissue of one-day old rat neonates, as well increasing body and kidney weight.

**References:**

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