Methyltrienolone influences on the androgen receptors and DNA & RNA synthesis in the liver cells of male rats with alloxan-induced diabetes

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Muntané Relat J.4,5 Padillo J.4,5

Abstract

Introduction: It has been revealed previously, that metabolic imbalances during the diabetes, occurring with multi-organ participation, are accompanied by the decrease of corticosterone concentration in blood and with the depression of DNA & RNA synthesis, and as well as expression of androgen receptors in liver cells.

Aim: The purpose of our study was to examine how exogenous testosterone can correct mentioned liver cell disturbances under the conditions of experimental diabetes.

Methods: The concentrations of glucose, immune-reactive insulin and testosterone were examined in blood in parallel with studying of histology of pancreatic islets on the 15th, 30th and 45th days from intraperitoneal injection of Alloxan - to confirm the development of adequate model of experimental (alloxan-induced) Diabetes in Wistar Rats aged 2 months and weighting 180-200 g. DNA and RNA synthesis, proliferative activity and androgen receptor expression in the hepatic cells were studied on the same terms of the experiment – to confirm the involvement of liver in diabetes-caused metabolic disturbances. All above-mentioned investigations were repeated in animals undergone to exposure of exogenous synthetic androgen – methyltrienolone during 15 days – beginning from 31st day of Alloxan-induced diabetes.

Results: Methyltrienolone supplementation reduces the alterations of blood concentration of immune-reactive insulin, glucose and testosterone and supports the regeneration of androgen receptors’ expression and DNA/RNA synthesis in hepatocytes. However, the increase of nuclear acids synthesis is accompanied by increase of hepatocytes ploidy but not their proliferation.

Conclusion: The administration of methyltrienolone reduces the effect of “diabetic stress”. Its influence on DNA/RNA synthesis in hepatocytes might be realized through the regeneration of active androgen receptors of liver cells. TCM-GMJ May 2017; 2(1):P10-P13

Keywords: Alloxan-induced diabetes, Testosterone, Liver androgen receptors, DNA, RNA, Pancreatic β-cells disturbance

Introduction

Intraperitoneal injection of Alloxan in male rats provokes the long-term injury of pancreatic islets followed by chronic hyperglycemia and generalized metabolism disorders. This model of experimental diabetes is widely used for investigation of different pathways based on multi-organ involvement and causing various complications of diabetes. Deterioration of sex hormones and liver metabolism is one of the well-known features of both types (type 1 and type 2) of diabetes in common and Alloxan-induced experimental diabetes, particularly.

The reduction of blood testosterone leads to the changes of metabolic processes in liver1,2 and alteration of DNA and RNA synthesis3. It is considered, that the functions of liver in males are changed more dramatically confirming the assumption that the sex-dependence (sex-association) of male livers are genetically more determined in compare with female livers4 Considering, that hepatocytes as well as β-cells of pancreatic islets are widely provided by the receptors of sex hormones, and particularly by androgen receptors, it may be proposed that testosterone supplementation may impact the hepatocytes and

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pancreatic β-cells metabolism via the pathways developing with involvement of these receptors.

The aim of the present study was to investigate how the exogenous androgen supplementation could influence on the expression of androgen receptors and DNA&RNA synthesis of liver cells impaired in alloxan-induced diabetes model.

**Material and Methods**

The model of alloxan-induced diabetes was developed in 80 male Wistar rats weighting 180-200 g. Administration of alloxan produced by Chemos GmbH & Co. KG (Germany) was performed as it was described before. 4 target groups with 20 animals in each one were created: the 1st group was studied on 15th day after alloxan intraperitoneal injection; the 2nd group - after 30 days, the 3rd group - after 45 days, correspondingly; the 4th group was studied after exposure of exogenous synthetic androgen – methyltrienolone - during 15 days, beginning from 31st day after injection of Alloxan. The control group with 20 healthy animals received solvent. The concentrations of glucose, immune-reactive insulin and testosterone were examined in blood obtained from caudal vena cava in parallel with studying of histology of pancreatic islets and liver cells in all above-mentioned groups.

One hour before removing animals from the experiments, the intraperitoneal injection of 3,7x10⁴Bq radioactive androgen Methyltrienolone [17α-METHYL-3H] (R-1881) was performed for the assessment of amount of androgen receptors in liver (the method was described in details earlier). Paraffin sections of pancreas and liver (4 µm) stained by hematoxylin and eosin were studied to assess the structure of pancreatic islets and ploidy of hepatocytes. Additional liver tissue sections were obtained for assessing Ki-67 expression by immunohistochemistry. After deparaffinization and rehydration, endogenous peroxidase was blocked in hydrogen peroxide solution. For the antigen restoration, the samples were placed in 0.01 M citrate buffer (pH 6.0) and were heated in a microwave oven (1000 C at 600W) for 15 minutes. After incubation with bovine serum to avoid unspecific binding and blocking of non-specific bonds, mouse monoclonal antibody anti-rat Ki-67 diluted 1:50 (clone MIB-5; DAKO) was incubated for 1 hour at room temperature. For the detection of bound antibodies Novolink Polymer Detection System (Leica, Germany) was used, and visualized using diaminobenzidine (DAB, Leica) and counterstained with hematoxylin.

To determine the ploidy of hepatocytes, the computer program Image J was used. The nuclei of 500 hepatocytes were measured on the corresponding histology sections. Based on the obtained data the diagrams were built for the squares roughly similar to their size. The first peak corresponded to 2C, subsequent peaks - 4C (2C×2) and 8C (4C×2). Tissue samples (3x5x5 mm³) were taken from the central and peripheral areas of all liver lobes.

The part of these samples being weighed, homogenized and placed in scintillation fluid, were placed in a scintillation counter "Beta-2" (Scientific-Research Institute of Medical Industry at Russian Academy of Medical Sciences, Moscow, USSR) for determining of the number of androgen receptors (AR) in liver (counting the radioactive impulses).

The quantities of DNA and RNA in liver were studied by radioactive H³-thymidine and H³-uridine as it was described before.

The study corresponded to the principles of the Guide for the Care and Use of Laboratory Animals (NRC 2011) and approved by the Commission on Bioethics at Al. Natishvili Institute of Morphology, Iv. Javakhishvili Tbilisi State University.

The obtained data were compared by using t test. p values less than 0.05 were considered statistically significant. Statistical analysis was conducted using SAS 9.2 software.

**Results and Discussion**

The development of adequate model of experimental diabetes is confirmed by alteration of the pancreatic islets with destruction of the part of their β-cells - on all studied terms from Alloxan injection (Figure 1). The structural disorders in the islets are accompanied by decrease of blood concentration of Insulin and Testosterone and increase of Glucose.

A reliable decrease in insulin concentration is observed on the 15th and 30th days of alloxan-induced diabetes. As for the 45th day, its decrease is no longer reliable (Table N1). This may be related to gradual leveling of the deleterious effect of alloxan on the 45th day of the experiment and to the initiation of pancreatic β-cell regeneration.

The dynamics of insulin concentrations decrease is respectively reflected in the dynamics of increasing glucose concentration in blood: This increase is significant on the 15th and 30th days of the experiment, but on the 45th day the increase of glucose concentration is no longer statistically reliable compared to the previous period (Table N1).

The decrease of the testosterone concentration was observed in the presence of the experimental diabetes, the dynamics of which is directly correlated with the dynamics of insulin concentration decrease: Testosterone concentration is decreased by 34.7% on the 15th day of the experiment, by 47.9% on the 30th day, and by 48.7% on the 45th day in comparison with the control data. These data are consistent with the results of other authors.

Hypoinsulinemia and decreased glucose utilization is accompanied by inhibition of DNA and RNA synthesis in hepatocytes. DNA synthesis was decreased by 44% and RNA synthesis by 29% at 15th day of the experiment which might be related with increased production of hydroxyl radicals, increasing the amount of Bel-2-associated X protein (BAX-protein), stimulating the release of cytochrome C from mitochondria with activation of caspase-3 dependent pathway of apoptosis.
(although, do not reach the norm figures), but do not change the concentration of insulin in the blood (it does not differ from the data of the 30th and 45th days of alloxan-induced diabetes). In addition, the concentration of glucose is significantly reduced and statistically is no longer different from the data of the 15th day of the experiment.

Exogenous androgen supplementation significantly increases DNA (24%) and RNA (8%) synthesis in liver followed by a raise on the amount of polyploid hepatocytes in compare with 30th and 45th days of AD model (Figure 2), although no increase in the number of Ki-67 positive hepatocytes was observed.

At the 15th day of alloxan-induced diabetes it was observed an increase of hepatic AR expression (Table N 1 and Diagram 2). As we previously supposed, this can be explained by down-regulation of testosterone and increased number of free AR enabled to interact with radioactive androgen (H3-methyltrionolone). The further deficit of testosterone provokes violent down-regulation of AR expression and signaling. Methyltrienolone supplementation leads to upregulation and activation of ARs, which in their turn, bind to radioactive H3-methyltrienolone (see above Materials and Methods) and increase in AR expression in liver cells.

Our study provides the additional data, that administration of methyltrienolone reduces the “stress” of alloxan-induced diabetes in liver cells. The intensification of DNA/RNA synthesis in hepatocytes by methyltrienolone might be realized through the regeneration of active androgen receptors of liver cells.

### Table 1. Blood concentration of glucose, immunoreactive insulin (IRI), testosterone (T) in rats; activity of nucleic acids (DNA and RNA) synthesis; expression of androgen receptor (AR); the hepatocytes ploidy.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose mmol/L</th>
<th>IRI µUu/mL</th>
<th>T ng/ml</th>
<th>DNAImp/g/min</th>
<th>RNA min</th>
<th>Imp/g/min</th>
<th>AR Imp/g/min</th>
<th>The hepatocyte ploidy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>5.05 ± 0.19</td>
<td>22.12 ± 1.36</td>
<td>2.65 ± 0.09</td>
<td>180849.63 ± 47.19</td>
<td>324904.44 ± 72.11</td>
<td>1676.45 ± 65.13</td>
<td>55.6/31.7/1.1</td>
</tr>
<tr>
<td></td>
<td>Diabetes (15th day)</td>
<td>11.84 ± 1.17</td>
<td>12.44 ± 0.69</td>
<td>1.73 ± 0.07</td>
<td>101289.42 ± 69.58</td>
<td>230694.17 ± 28.32</td>
<td>1888.25 ± 22.35</td>
<td>61.7/24.3/0.7</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>&lt;0.0001*</td>
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<tr>
<td>III</td>
<td>Diabetes (30th day)</td>
<td>13.22 ± 0.66</td>
<td>5.01 ± 0.42</td>
<td>1.38 ± 0.06</td>
<td>121163.57 ± 9.66</td>
<td>259955.76 ± 11.07</td>
<td>1124.98 ± 22.83</td>
<td>72.3/18.2/0.5</td>
</tr>
<tr>
<td></td>
<td>p value</td>
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<tr>
<td>IV</td>
<td>Diabetes 45th day</td>
<td>15.14 ± 0.62</td>
<td>4.75 ± 0.24</td>
<td>1.36 ± 0.09</td>
<td>106696.59 ± 16.44</td>
<td>243699.73 ± 20.63</td>
<td>1004.96 ± 8.57</td>
<td>77.3/10.7/0.2</td>
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<td>p value</td>
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<tr>
<td>V</td>
<td>Methyltrienolone supplementation during 15 days from 31th day of Diabetes</td>
<td>11.29 ± 0.83</td>
<td>4.79 ± 0.32</td>
<td>2.40 ± 0.08</td>
<td>150097.07 ± 12.95</td>
<td>282693.73 ± 20.63</td>
<td>1507.78 ± 10.51</td>
<td>59.2/25.5/0.9</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>&lt;0.0001*</td>
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Fig1. Disrupted normal architecture of pancreatic islets on 15th (a), 30th (b) and 45th (c) days of alloxan-induced Diabetes.

- Necrosis of β-cell - △;
- swelling of the intercellular substance - ▼;
- hypertrophy and vacuolization of β-cells - ▲;
- β-cells nuclear pyknosis (irregular hyperchromic nuclei) - ▲;
- cytoplasmic degenerative changes in center of the islet - ▲;
- apparent decrease in cell density - ▼;

Fig2. Ploidy of hepatocytes at 30th day of alloxan induced diabetes (a, b), and at 15th day of exogenous androgen supplementation (c, d).

diploid nuclei - △
tetraploid nuclei - ▲

References


