Histological Characteristics of Thymus Assessed with Stereological Parameters after Medroxyprogesterone Acetate Application

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Abstract

Aim: The purpose of this research was to examine the effect of the synthetic progestin medroxyprogesterone acetate (MPA) on the morphological characteristics of the thymus.

Material and Methods: A total of 24 female Wistar rats were divided into two groups. The control group received saline, and the second group was administered medroxyprogesterone acetate in the therapeutic dose of 30 mg/kg bw. The substances were applied daily, intramuscularly for a period of 7 days. Paraffin sections of thymus were dyed with the following methods: hematoxylin - eosin and elastica Van Gieson.

Results: Histological analysis of the samples obtained after the application of MPA, showed a reduction in the thymic parenchyma and increasing of the stroma. Stereological analysis and statistical data processing (Student t-test) showed that the volume density (% per mm³ tissue) of thymic parenchyma was 89.94 ± 0.85% (average value ± standard deviation) in the control group of rats, while significantly decreased to 18.07 ± 2.20% in the group of rats treated with MPA (p <0.01). It was due to significant reduction of the cortex (from 71.37 ± 1.33% to 11.81 ± 1.31%, p <0.01) and significant reduction of the medulla (from 18.56 ± 1.07% to 6.23 ± 0.93%, p <0.01). To account thymic parenchyma, intense presence of increased stroma and its bulk density of 10.06 ± 0.85% in control group of rats significantly increased to 81.93 ± 2.20% after the treatment with MPA.

Conclusion: The results showed that major morphological characteristic of the thymus is the atrophy of its parenchyma after the application of medroxyprogesterone acetate.

Introduction

Scientific knowledge about the effect of synthetic progestin medroxyprogesterone acetate (MPA) on the immunological system shows contrasting attitudes. Most of the data on MPA immunosuppressive effect have been obtained from examinations of patients with advanced cancer of the endometrium treated with hormone therapy with this progestin or during the treatment of lymph node metastasis, after surgery for primary breast cancer [1, 2]. Although the adverse drug reactions are controllable or tolerable, the most common reported effects that appear after this kind of therapy are leucopenia [3-5], suppression of bone marrow [6], suppression of T – lymphocytes [7] and regression of lymph nodes [8]. There are some authors who believe...
that progestins have no distinct immunosuppressive effect [9, 10], that is, progestins have no significant influence on immunologic organs [11]. However, the question on the immunosuppressive effect of MPA still remains open since in the clinical practice of some medical institutions MPA is used as immunopotentiator, whereas some researchers think that it has no important influence on the immunologic organs [12].

The aim of the work was to determine the effect of MPA on thymus morphology by determining volume density of thymic structural components: parenchyma (cortex and medulla) and stroma.

**Material and Methods**

A total number of 24 female Wistar rats were divided into two groups, each one containing 12 animals. The first, control group of rats was given physiological solution and the second, experimental group of rats was administered MPA in a dose of 30 mg/kg bw. The substances were given by intramuscular application every day, with a volume of 0.1 ml in a period of 7 days.

The animals were sacrificed 24 hours after application of the MPA last dose under ether anesthesia. Then, the extracted thymuses were fixed in 10% buffered formalin, and paraffin sections were stained according to the methods of hematoxylin – eosin and elastica Van Gieson. Histological (qualitative) and stereological (quantitative) analyses were performed by using light microscope. Ocular with built in Weibel’s multipurpose test system (M – 42) was used for stereological analysis. Volume density of parenchyma (cortex and medulla) and interstitial connective tissue of the thymus were determined according to the following formula:

\[ V_v = \frac{P_f}{P_t} \]

Where: \( V_v \) is volume density of the examined phase, a relative stereological value that shows the total space occupied by the examined phase. If the obtained value is multiplied with 100, the result will show the percent of the examined phase per volume unit. The number of reference fields where we performed the stereological measurements was at least 100 for each thymus. \( P_f \) is the number of spots that fall on the examined phase. \( P_t \) is the total number of spots of the test system.

The values of the volume density give the basic data for the structure of the examined organ or tissue.

Quantitative data obtained from the stereological analysis were processed with the statistical method Student’s t – test.

**Results**

Qualitative histological analysis has shown that the thymus obtained from the control group of rats has normal histological structure. Both the capsule and trabeculae are with common thickness. The lobules are with approximately similar size and they are clearly differentiated into a cortex and medulla. Thymic cortex is characterized with usual lymphocyte density. Presence ratio between parenchyma and stroma in the thymus has revealed that the greatest part of thymic mass belongs to the parenchymal tissue. However, the ratio between cortex and medulla in the thymic lobules goes in favor of the cortex (Fig. 1).

Figure 1: Control group: characteristic structure of the thymus; capsule, trabeculae and lobules with clearly differentiated cortex and medulla and usual density of lymphocytes; elastica Van Gieson, 10 X 4.

In the animals treated with MPA disorder of the regular lobulation of the parenchyma is noticed as well as obvious decrease of the thymus lobules, mainly as a result of the decrease in the presence of thymic parenchyma (Fig. 2).

Reduction of the cortex thickness has been registered with evident decrease of the density of the lymphocytes (Fig. 3).

At some sites cortex is even absent. Stroma presence is intensively increased as a result of the increase of both interlobular and intralobular connective tissue (Fig. 4).
Quantitative stereological analysis has shown that volume density of parenchyma is significantly reduced and volume density of stroma is significantly increased after MPA administration. Presence ratio between volume density of parenchyma and stroma is 8.9 : 1 (in favor of parenchyma), which was found in the control group of rats. It has been strikingly changed to 4.5:1 (in favor of stroma) in the group of rats treated with MPA. The reduction of parenchyma is due to the reduction of the volume density in its both structural components, cortex and medulla. However, the degree of their decrease is different. The ratio between volume density of the cortex and medulla is 3.8 : 1 in the control group of rats; it has decreased and reached 1.9 : 1 in the rats treated with MPA. The data for the volume density of the thymic structural components and their statistical processing are presented in Table 1.

### Discussion

Normal morphology with normal volume density of parenchyma, that is, functional, lymphocyte cellular compartment is a morphological indicator for normal, functional condition of the thymus. On the other hand, reduction of the volume density of parenchyma implies to a decreased function that is, condition of immunosuppression.

### Table 1: Volume density (% per mm³ tissue) of parenchyma and stroma of the thymus and volume density of cortex and medulla of the thymus lobules

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>MPA (30 mg/kg bw)</th>
<th>t-value</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parenchyma</td>
<td>X₁ ± SD</td>
<td>X₂ ± SD</td>
<td>105.45</td>
<td>22</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Stroma</td>
<td>X₁ ± SD</td>
<td>X₂ ± SD</td>
<td>105.45</td>
<td>22</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cortex</td>
<td>X₁ ± SD</td>
<td>X₂ ± SD</td>
<td>110.62</td>
<td>22</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Medulla</td>
<td>X₁ ± SD</td>
<td>X₂ ± SD</td>
<td>30.08</td>
<td>22</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

df – degree of freedom; SD – standard deviation; X₁ – mean of the volume density of variables in the control group of rats; X₂ – mean of the volume density of variables in the group of rats treated with MPA.

In the investigation was assessed the immunomodulator effects of MPA on the thymus by determining volume density of thymic principal compartments: parenchyma (cortex and medulla) and stroma.

The results have shown that crucial change in the thymus morphology provoked by MPA is decreased...
of the volume density of parenchyma and increase of the volume density of stroma. This change speaks in favor of the reduction of the functional, lymphocyte cellular potential, that is, immunomodulation towards immunosuppression. It is assumed that one of the reasons for decrease of the volume density of parenchyma may be the slow generation or interruption in the production of lymphocytes, that is, inhibition of blastogenic response of the lymphocytes.

The first data on the effect of MPA on lymphocyte blastogenic response to mitogen substances and information obtained from the examinations with E – roset’s test were published by Corsini G [13]. He showed that MPA has a significant, inhibitory effect only if it is used in definite concentration. In other investigations some contrary findings have been presented indicating that in spite of the inhibition of the proliferative responses, there is increase of the accumulation of immunoglobulin secreting cells in the cultures of tissues stimulated with mitogen substances. However, some authors affirm that MPA did not increase the number of immunoglobulin secreting cells, because their findings suggest that this progestin enhanced the capacity of individual cells to produce specific immunoglobulin [14].

Quantification of the inhibitory effects has been performed by determination of the percent of reduction of thymidine incorporation in DNA in sheep’s peripheral blood lymphocytes. It has been shown that MPA reduces thymidine incorporation in DNA for about 24% [15]. Some authors registered decreased nitric oxide production in isolated leucocytes. These investigations designate the immunosuppressive effects of MPA [16].

Some findings have pointed that MPA can severely reduce the blastogenic response [17]. The most recent investigations have confirmed previous knowledge that MPA provokes apoptotic changes of T lymphocytes in the lymph nodes [18]. Despite multiple contradictory experimental studies, clinical observations suggest that MPA inhibits CD8+ T cell viral specific effector function and induces herpes simplex virus type 1 reactivation. This fact again confirms the immunosuppressive effect of MPA [19].

Increase of the volume density of stroma has been registered in our examination. This finding presents a morphological indicator for the greater resistance of the stroma in comparison with the parenchymal tissue of the thymus.

The number of morphological studies on immunomodulator effect of progestins (including MPA) is very small and their findings are controversial.

According to some authors progestins do not provoke atrophy or any other morphological abnormality of the spleen and thymus, that is, lymphoid system remains morphologically unchanged [20]. There are some authors who believe that MPA has immunostimulating effect that cannot be noticed since it is masked by the endogenous glucocorticoids [21].

We published histological analysis of the spleen which showed an obvious reduction of the lymph follicles which were in an involuntary phase with inactive germinal centers. Destructive changes in tissue were registered in the close distance of which a more intensive development of connective tissue was noticed into which collagen fibres predominate [22]. Histological analysis demonstrated that MPA caused the following morphological changes in adrenal cortex: more intensive development of stroma; decrease of adrenal cortex thickness; disturbance of spacial organization of adrenocorticocytes in glomerular zone, fascicular zone and reticular zone; atrophic changes of adrenocorticocytes; disappearance of intermediary zone after application of 75.0 mg/kg bw MPA; appearance of microcysts in fascicular and reticular zones; decrease of cortical proliferate dimensions and accessory adrenal glands; disappearance of spongiocytes from their structure and atrophic changes of glomerular cells; decrease of adrenal cortex vascularisation, necrotic changes localized subglomerularly and in fascicular zone of adrenal cortex [23].

The quantitative histological analysis showed significant decrease of the adrenal cortex, i.e. decrease of glomerular, fascicular and reticular zone thickness and significant decrease of the adrenocorticocytes nuclei volume, changes which suggested that MPA caused an atrophy of the adrenal cortex [24].

However, other authors think that MPA causes significant reduction of the spleen weight due to the atrophy of its lymphoid tissue and obvious reduction of the lymph nodes.

The results of our investigation have shown that after application of MPA the presence ratio of parenchyma and stroma is significantly changed in favor of the stroma, which emphasizes the fact that the major morphological characteristic of thymus after application of MPA is atrophy of its parenchyma.
References


