

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

Elida Mitevska

Institute of Medical and Experimental Histology and Embryology, Medical Faculty, University "Ss Cyril and Methodius", 50 Divizija 16, Skopje, Republic of Macedonia

Abstract

Citation: Mitevska E. Histological Characteristics of Thymus Assessed with Stereological Parameters after Medroxyprogesterone Acetate Application. 2011 Dec 15; 4(4):367-371. Maced J Med Sci. <http://dx.doi.org/10.3889/MJMS.1957-5773.2011.0197>.

Key words: medroxyprogesterone acetate (MPA); thymus; histology; stereology; Wistar rats.

Correspondence: Dr. Elida Mitevska, MD, PhD. Institute of Medical and Experimental Histology and Embryology, Faculty of Medicine, University "Ss Cyril and Methodius", 50 Divizija No 16, Skopje 1109, Republic of Macedonia. E-Mail: elida_mitevska@yahoo.com

Received: 27-Sep-2011; Revised: 24-Oct-2011; Accepted: 26-Oct-2011; Online first: 07-Nov-2011

Copyright: © 2011 Mitevska E. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Competing Interests: The author have declared that no competing interests exist.

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 syntetic 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:

$$V_{vf} = P_f/P_t$$

Where: V_{vf} 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).

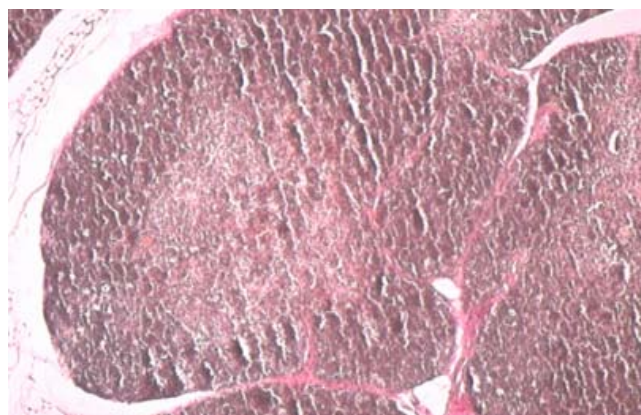


Figure 1: Control group: characteristic structure of the thymus; capsule, trabeculae and lobules with clearly differentiated cortex and medulla and usual density of lymphocytes; elastica Van Gieson, 10 X 4.

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).

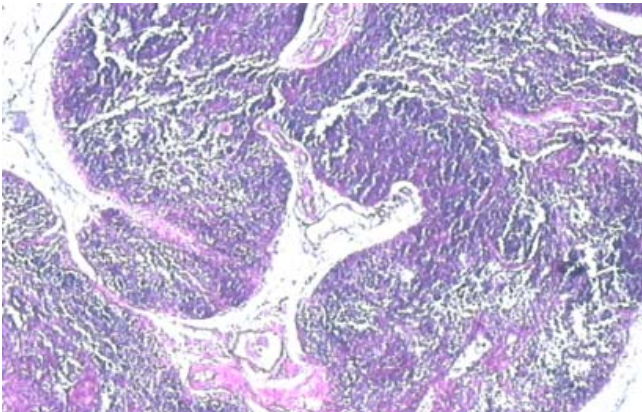


Figure 2: MPA, 30 mg/kg bw: disorder of the regular lobulation of the thymic parenchyma; hematoxylin – eosin, 10 X 4.

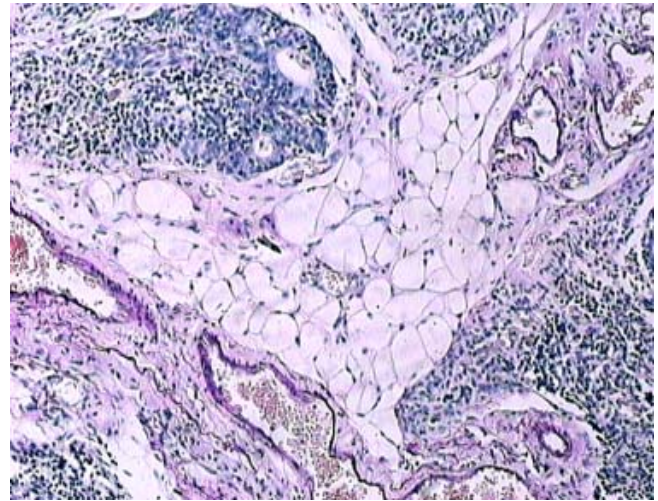


Figure 4: MPA, 30 mg/kg bw: lobules with reduced presence of parenchymal tissue, and intensive infiltration of stroma in the thymus; elastica Van Gieson, 10 X 10.

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.

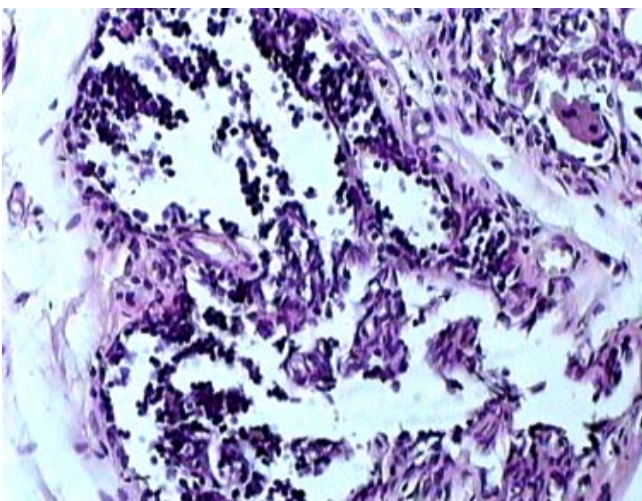


Figure 3: MPA, 30 mg/kg bw: decrease of the density of lymphocytes and appearing of evident lymphocyte depletion; hematoxylin – eosin, 10 X 20.

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.

Variable	n	Control group	MPA (30 mg/kg bw)	t-value	df	p
		X ₁ ± SD	X ₂ ± SD			
Parenchyma	12	89.94 ± 0.85	18.07 ± 2.20	105.45	22	< 0.01
Stroma	12	10.06 ± 0.85	81.93 ± 2.20	105.45	22	< 0.01
Cortex	12	71.37 ± 1.33	11.81 ± 1.31	110.62	22	< 0.01
Medulla	12	18.56 ± 1.07	6.23 ± 0.93	30.08	22	< 0.01

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

- Bafaloukos D, Aravantinos G, Samonis G, Katsifis G, Bakoyiannis C, Skarlos D, Kosmidis P. Carboplatin, methotrexate and 5-fluorouracil in combination with medroxyprogesterone acetate (JMF-M) in the treatment of advanced or recurrent endometrial carcinoma: a hellenic cooperative oncology group study. *Oncology*. 1999;56(3):198 – 201.
- Keichi M, Hiroshi K, Norimichi K. Treatment with doxifluridine, medroxyprogesterone acetate, and cyclophosphamide for supraclavicular lymph node metastasis from breast cancer. *Japanese Journal of Breast Cancer*. 2001;16(6):603 – 610.
- Kihara M, Kontani K, Yamauchi A, Yokomise H. Two cases of advanced breast cancer responding to oral chemoendocrine therapy with 5'-deoxy-5-fluorouridine, medroxyprogesterone acetate and cyclophosphamide (DMpC). *Gan To Kagaku Ryoho*. 2005;32(5):683 – 686.
- Iino Y, Yokoe T, Sugamata N, Maemura M, Takei H, Horiguchi J, Takeyoshi I, Ohwada S, Morishita Y, Kusaba T et al. A combination chemoendocrine therapy of mitoxantrone, doxifluridine, and medroxyprogesterone acetate for anthracycline-resistant advanced breast cancer. *Cancer Chemother Pharmacol*. 1998;41(3):243–247.
- Kimura M, Hagiwara S, Hirose K, Shimokawa Y, Iwai K, Uemura K. Two cases of advanced breast cancer effectively treated with chemoendocrine therapy and radiotherapy. *Gan To Kagaku Ryoho*. 1998;21(14):2505–2508.
- Yoshinaka K, Yagi M, Ohtagaki S, Toi M, Toge T. 5'-deoxy-5-fluorouridine (5'-DFUR), mitomycin C (MMC), etoposide and medroxyprogesterone acetate (MPA) in a previously treated patient with advanced breast cancer. *Gan To Kagaku Ryoho*. 1991;18(1):115–118.
- Bakhidze EV, Bokhman JaV. Application of thymic factor "Thymalin" in complex treatment of endometrial cancer patients. *Eur J Gynecol Oncol*. 1990;11(4):251–256.
- Yamada T, Okazaki M, Okazaki A et al. A case of inflammatory breast cancer treated with medroxyprogesterone acetate (MPA) in combination with intra-arterial infusion chemotherapy. *Gan To Kagaku Ryoho*. 1992;19(11):1923–1925.
- Gronroos M, Eskola J. In vitro functions of lymphocytes during high-dose medroxyprogesterone acetate (MPA) treatment. *Cancer Immunol Immunother*. 1984;17:218–220.
- Cetin M, Ozkul Y, Unal A, Eser B, Ozturk O, Kutlubay R, Er O, Burakgazi H. The effect of medroxyprogesterone acetate on bone marrow and testis during cytotoxic chemotherapy. *Cell Biology and Toxicology*. 2006;16(6):385–390.
- Selman PJ, Van Garderen E, Mol JA, Van den Ingh TS. Comparison of the histological changes in the dog after treatment with the progestins medroxyprogesterone acetate and proligestone. *Vet Q*. 1995;17(4):128–133.
- Kusama M, Kimura K, Aoki T et al. Complete remission, obtained by multidisciplinary treatment of recurrent breast cancer with carcinomatous pleuritis, and cervical lymph node and liver metastasis. *Gan No Rinsho*. 1989; 35(1): 93 – 99.
- Corsini G, Puppo F. Effect of medroxyprogesterone acetate upon PHA, Con A and PWM stimulated lymphocytes and on E-rosette function. *J Immunopharmacol*. 1982-83; 4(3): 247 – 253.
- Vermeulen M, Pazos P, Lanari C, Molinolo A, Gamberale R, Gefner JR, Giordano M. Medroxyprogesterone acetate enhances in vivo and in vitro antibody production. *Immunology*. 2001;104(1):80–86.
- Staples LD, Heap RB, Brown D, Marrs RWI. Structural requirements for steroid inhibition of sheep lymphocyte mitogenesis in vitro. *Steroids*. 1984;44(5):419–433.
- Pietsch C, Neumann N, Preuer T, Kloas W. In vivo treatment with progestogens causes immunosuppression of carp *Cyprinus carpio* leucocytes by affecting nitric oxide production and arginase activity. *Journal of Fish Biology*. 2011;79(1):53 – 69.
- Mantovani G, Maccio A, Esu S et al. Medroxyprogesterone acetate reduces the in vitro production of cytokines and serotonin involved in anorexia/cachexia and emesis by peripheral blood mononuclear cells of cancer patients. *Eur J Cancer*. 1997;33(4):602–607.
- Hayden RE, Pratt G, Drayson MT, Bunce CM. Lycorine sensitizes CD40 ligand – protected chronic lymphocytic leukaemia cells to bezafibrate– and medroxyprogesterone acetate– induced apoptosis but dasatinib does not overcome reported CD40–mediated drug resistance. *Hematologica*. 2010;95(11):1889–1896.
- Cherpes TL, Busch JL, Sheridan BS, Harvey SAK, Hendricks RL. Medroxyprogesterone acetate inhibits CD8⁺ T cell viral specific effector function and induces herpes simplex virus type 1 reactivation. *J Immunol*. 2008;181(2):969–975.
- Malarkey WB, Burleson M, Cacioppo JT, Poehimann K, Glaser R, Kiecolt-Glaser JK. Differential effects of estrogen and medroxyprogesterone on basal and stress-induced growth hormone release, IGF-1 levels, and cellular immunity in postmenopausal women. *Endocrine*. 1997;7(2):227–233.
- Hory Y, Hu DE, Yasui K, Smither RL, Gresham GA, Fan TP. Differential effects of angiostatic steroids and dexamethasone on angiogenesis and cytokine levels in rat sponge implants. *Br J Pharmacol*. 1996;118(7):1584–1591.
- Mitevska ES, Gerasimovska ZA, Kakaseva LS, Trajkov DK, Jurhar MR, Stojovska PE, Spiroski MZ. Histophysiology of the spleen after application of medroxyprogesterone. *Zbornik Matice srpske za prirodne nauke*. 1998;95:25-32.
- Mitevska E, Spiroski M. Quantitative Analysis of Adrenal Cortical Histological Alterations After Application of Medroxyprogesterone Acetate. *Maced J Med Sci*. 2010; 3(4):352-357.
- Mitevska E, Spiroski M. Qualitative Histological Analysis of Adrenal Cortex Following Application of Medroxyprogesterone Acetate. *Maced J Med Sci*. 2010;3(2):123-131.