

Quantitative Analysis of Adrenal Cortical Histological Alterations After Application of Medroxyprogesterone Acetate

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Abstract

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Aim. The aim of our investigation was to make a quantification of the changes being registered by a quantitative histological analysis of adrenal cortex after long-term application of high doses of medroxyprogesterone acetate (MPA).

Material and Methods. A total of 48 female Wistar rats were divided into four groups. The control group was given saline intramuscularly, every day, and the other three groups MPA in doses of 7.5, 15.0 and 75.0 mg/kg bw, respectively, during 30 days. Paraffin sections from adrenal glands were stained according the following methods: hematoxylin-eosin, Azan, modified by Heidenhein, and Masson.

Results. Gravimetric and variance analysis demonstrated the most remarkable decrease of the adrenal gland weight after the application of 75.0 mg/kg/bw MPA (from 35.66 ± 11.78 to 13.41 ± 8.41 mg ($p < 0.001$)). On the other hand, morphometric analysis demonstrated that adrenal cortex volume was the most significantly reduced from 11.30 ± 3.53 in the control group to 2.04 ± 0.90 mm³ in the group treated with 15.0 mg/kg bw MPA ($p < 0.0001$). Adrenal cortex thickness was reduced from 846.06 ± 6.48 to 349.55 ± 4.84 μ m after the application of 75.0 mg/kg bw ($p < 0.001$). After the application of the same dose the glomerular zone thickness was decreased from 48.02 ± 0.44 to 41.40 ± 0.43 μ m ($p < 0.001$), the fascicular zone thickness from 480.72 ± 3.43 to 194.32 ± 2.80 μ m ($p < 0.001$) and the reticular zone thickness reduced from 317.47 ± 4.80 to 113.85 ± 2.28 μ m ($p < 0.001$). The volume of glomerular zone cell nuclei decreased most remarkably from 94.79 ± 0.96 μ m³ after the application of 75.0 mg/kg bw to 60.12 ± 0.88 μ m³ ($p < 0.001$). The volume of fascicular zone cell nuclei decreased from 166.30 ± 1.53 to 52.47 ± 0.89 μ m³ ($p < 0.001$), while the volume of reticular zone cell nuclei reduced from 95.61 ± 1.03 to 47.68 ± 0.92 μ m³ ($p < 0.001$). The analysis by means of χ^2 test showed that the appearance of tissue necrotic changes in adrenal cortex had a statistical significance only after the application of the highest dose of MPA ($p < 0.012$).

Conclusion. 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.

Introduction

Potent synthetic progestin medroxyprogesterone acetate (MPA) which suppresses the pituitary-gonadal axis, suppresses also the pituitary-adrenal axis [1, 2].

However, the reports concerning the size the

pituitary-adrenal axis has been involved, are not identical. In majority of experimental animals (rats, monkeys, ground squirrels) MPA caused adrenal atrophy and exhaustion of pituitary ACTH concentrations [3-6].

As for the investigation on human population,

various effects have been recorded caused by MPA. Although minority of researchers considered that MPA had no significant effects on pituitary-adrenal axis, however majority of authors recorded remarkable adrenal suppression. The use of MPA for contraceptive purposes in usual doses, did not cause significant effect on adrenal cortex [7], but when applied in high doses, the MPA effect was proved on adrenal glands besides the remarkable effect on gonads [2, 8-13]. In majority of patients with endometrial or breast cancer, conditions in which MPA is used in high doses for a longer period of time [14-16], significant decrease of plasmatic cortisol and ACTH concentrations was observed after 4 to 8 weeks of the MPA treatment, i.e. potent MPA effect on pituitary-adrenal axis was found [9].

In our study for histochemical changes of the adrenal cortex after the application of MPA the following changes were recorded: more intensive development of stroma; reduction of adrenal cortical thickness; disturbance of adrenocorticocytes spatial organisation in glomerular, fascicular and reticular zones, adrenocorticocyte atrophic changes; disappearance of intermediary zone after the application of 75.0 mg/kg bw MPA; appearance of microcysts in fascicular and reticular zones; reduction of dimensions of cortical proliferates and accessory adrenal glands; disappearance of spongiocytes from their structure and atrophic changes of glomerulosa-cells; reduction of vascularization of adrenal cortex, necrotic changes localized subglomerularly and in fascicular zone from the adrenal cortex [17]. As for the quantitative evaluation, the MPA effect on pituitary-adrenal axis has been mainly evaluated on the basis of the following quantitative parameters: the adrenal gland weight, the pituitary weight, plasmatic cortisol value, diurnal cortisol production (after maximal ACTH stimulation) and the plasmatic ACTH value [18].

Regarding the data obtained by quantitative histological analysis, there were investigations in which only the adrenal cortical thickness was determined [4, 19], and the number of the cells in "single area" of fascicular and reticular zones in adrenal cortex [3]. Because of this, our aim was to make quantification of the changes which were recorded by quantitative histological analysis of adrenal cortex after application of high doses of MPA.

Material and Methods

The experiment was made on 48 female Wistar

rats, divided into 4 groups. The first, control, group of rats was given saline every day (0.1 mL), intramuscularly within 30 days. The other three groups of rats were treated by MPA (Dugan forte, Inex Hemofarm, Vrsac, Serbia) in three different doses: 7.5, 15.0, and 75.0 mg/kg bw. Paraffin sections from adrenal glands were stained after the methods: hematoxylin-eosin, Azan, modified by Heidenhein, Van-Gieson, Kossa-Goldner, Masson and Florentin. Then, for quantitative analysis the following methods were used: morphometry (quantitative histologic analyses) and gravimetry (measurement of the adrenal gland by means of rotatory scale). Eyepiece with 10X enlargement, with built-in micrometer ruler with 100 length divisions was used for morphometric analysis. Depending on the parameter measured, lenses for 4X, 10X and 100X enlargements were used. Sections from the medial parts of the adrenal glands were separated for measurement of the adrenal glands diameters, the adrenal medulla diameters, cortex thickness and its zones. Moreover, the longest and the shortest diameters of the adrenal gland and the longest and the shortest diameters of adrenal medulla were determined. After determination of the adrenal gland volume and adrenal medulla volume, the adrenal cortex volume was determined indirectly.

From each adrenal gland, individual zones thickness was measured in 40 different, randomly chosen spots, so for each of these parameters in the experimental group 480 measurements were obtained. After that, volumes of 100 nuclei from each zone, from each adrenal gland with previously measurement of the longest and the shortest diameters of each nucleus were calculated. In this way, in every group the volumes of 1200 nuclei from each separate zone were determined. Size of all dimensions of the parameters measured was corrected by the factor which value was obtained when checking the microscope. Variance analysis and Newman-Keul test for multiple comparisons were used for statistical calculation of quantitative data. χ^2 - test was used for statistical analysis of the qualitative data.

Results

Statistical analysis for the adrenal gland weight using the variance analysis demonstrated different decrease of the gland weight depending on the dose used. After the application of 7.5 mg/kg bw MPA the adrenal gland weight decreased from 35.66 ± 11.78 mg (mean value \pm standard deviation), as it was found in the rats control group, to 25.41 ± 24.02 mg. Extremely remark-

able decrease of the adrenal gland weight was found after the application of 15.0 mg/kg bw MPA, and it was 13.5 ± 3.63 mg, while after the application of 75.0 mg/kg bw MPA it was 13.41 ± 8.41 ($F = 6.89$; $p < 0.001$) (Table 1).

The Newman-Keul test for multiple comparison showed significant decrease of the adrenal gland weight after the application of both higher doses compared to the adrenal gland weight after the treatment with 7.5 mg/kg bw MPA. Comparison of the adrenal gland volume mean value showed significant decrease of this parameter; from 12.00 ± 1.46 mm³ (mean value \pm standard deviation) as it was in the rats control group, it significantly decreased to 2.96 ± 1.45 mm³ after the application of 7.5 mg/kg bw MPA; 2.53 ± 1.01 mm³ after the application of 15.0 mg/kg bw MPA; and 2.49 ± 0.95 mm³ after the application of 75.0 mg/kg bw MPA ($F=55.53$; $p < 0.0001$) (Table 1).

Mean value of the adrenal gland medulla volume demonstrated slight decrease under the influence of MPA, however, the variance analysis showed that this change was not statistically significant. The analysis of the adrenal cortical gland volume demonstrated significant decrease after the application of all three doses of MPA. Adrenal cortical volume in rats control group was

Table 1: Variance analysis of the adrenal gland weight and volume, medulla volume, and adrenal cortical gland volume.

Parameter	n	Saline	7.5 mg/kg bw MPA	15 mg/kg bw MPA	75 mg/kg bw MPA	F	P
Adrenal gland weight (mg)	12	$35.66 \pm 1.78^*$	25.41 ± 24.02	13.5 ± 3.63	13.41 ± 8.41	6.89	< 0.001
Adrenal gland volume (mm ³)	12	12.00 ± 3.84	2.96 ± 1.45	2.53 ± 1.01	2.49 ± 0.95	55.53	< 0.0001
Medulla volume (mm ³)	12	0.69 ± 0.37	0.51 ± 0.38	0.49 ± 0.17	0.42 ± 0.19	1.81	NS
Cortex volume (mm ³)	12	11.30 ± 3.53	2.45 ± 1.13	2.04 ± 0.90	2.07 ± 0.81	65.5	< 0.0001

*. Each value is arithmetic mean value \pm standard deviation of the number of cases; F, variance analysis; p, significance of the differences among the group investigated; n, number of cases; bw, body weight; MPA, medroxyprogesterone acetate; NS, non-significant.

11.30 ± 3.53 mm³ while after the application of 7.5 mg/kg bw MPA it was 2.45 ± 1.13 mm³; after the application of 15.0 mg/kg bw: 2.04 ± 0.90 mm³ and after application of 75.0 mg/kg bw of MPA: 2.07 ± 0.81 mm³ ($F=65.6$; $p < 0.0001$) (Table 1).

Mean value of the adrenal cortical thickness in rats control group accounted for 846.06 ± 6.48 μ m (mean value \pm standard error). After the application of 7.5 mg/kg bw of MPA the adrenal cortical thickness was 370.96 ± 4.43 μ m; after the application of the dose of 15.0 mg/kg bw, the parameter measured was 370.23 ± 3.76 μ m; and after the application of 75.0 mg/kg bw the adrenal cortical thickness represented 349.55 ± 4.84 μ m

($F=999.99$; $p < 0.001$) (Table 2).

Statistical analysis of the glomerular zone thickness demonstrated that the thickness of this zone was not significantly changed after the application of MPA in dose of 7.5 mg/kg bw. But, after the management of 15.0 mg/kg bw of MPA its thickness, from 48.02 ± 0.44 μ m, as it was in the rats control group, significantly decreased to 44.66 ± 0.46 μ m after the application of 15.0 mg/kg bw of MPA and 41.40 ± 0.43 μ m after application of 75.0 mg/kg bw ($F=32.57$; $p < 0.001$) (Table 2).

Fascicular zone thickness significantly decreased from 480.72 ± 3.43 μ m (control group) to 218.51 ± 3.00 μ m (experimental group of rats treated with 7.5 mg/kg bw); 211.71 ± 2.56 μ m (experimental group of rats treated with 15.0 mg/kg bw of MPA) and 194.32 ± 2.80 μ m (experimental group of rats treated with 75.0 mg/kg bw of MPA) ($F=999.99$; $p < 0.001$) (Table 2).

Reticular zone thickness also significantly decreased after the treatment with all three doses of MPA compared to the control. The mean value of the reticular

Table 2: Variance analysis of the adrenal cortical gland thickness and its zones: glomerular, fascicular and reticular zones.

Parameter	n	Saline	7.5 mg/kg bw MPA	15 mg/kg bw MPA	75 mg/kg bw MPA	F	P
Adrenal cortical gland thickness (μ m)	480	$846.06 \pm 6.48^*$	370.96 ± 4.43	370.23 ± 3.76	349.55 ± 4.84	999.99	< 0.001
Thickness of glomerular zone (μ m)	480	48.02 ± 0.44	48.25 ± 0.82	44.66 ± 0.46	41.40 ± 0.43	32.57	< 0.001
Thickness of fascicular zone (μ m)	480	480.72 ± 3.43	218.51 ± 3.00	211.71 ± 2.56	194.32 ± 2.80	999.99	< 0.001
Thickness of reticularis zone (μ m)	480	317.47 ± 4.80	104.19 ± 1.99	114.35 ± 2.01	113.85 ± 2.28	999.99	< 0.001

*. Each value is arithmetic mean value \pm standard error of the number of cases; F, variance analysis; p, significance of the differences among the group investigated; n, number of cases; bw, body weight; MPA, medroxyprogesterone acetate.

zone thickness in rats control group was 314.47 ± 4.80 μ m. After the application of 7.5 mg/kg bw of MPA it was 104.19 ± 1.99 μ m; after the application of 15.0 mg/kg bw MPA its value was 114.34 ± 2.01 μ m; after the application of 75.0 mg/kg bw of MPA: 113.85 ± 2.28 μ m; ($F=999.99$; $p < 0.001$) (Table 2).

Determination of the glomerular zone cell nuclei volume demonstrated significant decrease of this parameter in all groups of rats treated by MPA. Nuclei volume of glomerulosa-cells in rats control group was 94.79 ± 0.96 mm³ (mean value \pm standard error). After the application of 7.5 mg/kg bw of MPA this parameter reduced to 67.59 ± 0.88 mm³; usage of 15.0 mg/kg bw of MPA caused volume decrease to 61.93 ± 0.81 mm³; and the usage of 75.0 mg/kg bw caused the most significant

decrease of the nuclei volume and it was $60.12 \pm 0.88 \mu\text{m}^3$; ($F=329.29$; $p < 0.001$) (Table 3).

Variance analysis demonstrated that the greatest atrophic changes, i.e. the most prominent decrease were found in the fascicular zone cell nuclei volume (spongiocytes). Drastic and significant volume decrease of spongiocyte nuclei was observed after the application of all three doses of MPA. Mean value of the cell nuclei volume in fascicular zone in the rats control group from was $166.30 \pm 1.53 \mu\text{m}^3$. Mean value of the spongiocyte nuclei volume after the treatment with 7.5 mg/kg bw of MPA was $58.02 \pm 0.83 \mu\text{m}^3$; after the treatment of 15.0 mg/kg bw it was $56.01 \pm 0.84 \mu\text{m}^3$; and after the treatment with 75.0 mg/kg bw this parameter had the lowest value: $52.47 \pm 0.89 \mu\text{m}^3$ ($F=999.99$; $p < 0.001$) (Table 3).

Reticular zone cell nuclei volume significantly decreased as well after the treatment of the rats with all

Table 3: Variance analysis of the adrenocorticocyte nuclei volume in glomerular, fascicular and reticular zones.

Parameter	n	Saline	7.5 mg/kg bw MPA	15 mg/kg bw MPA	75 mg/kg bw MPA	F	P
Glomerular zone cell nuclei volume (μm^3)	600	$94.79 \pm 0.96^*$	67.59 ± 0.88	61.93 ± 0.91	60.12 ± 0.88	329.29	< 0.001
Fascicular zone cell nuclei volume (μm^3)	600	166.30 ± 1.53	58.02 ± 0.83	56.01 ± 0.84	52.47 ± 0.89	999.99	< 0.001
Reticularis zone cell nuclei volume (μm^3)	600	95.61 ± 1.03	53.58 ± 0.89	49.75 ± 0.85	47.68 ± 0.92	600.53	< 0.001

*. Each value is arithmetic mean value \pm standard error of the number of cases; F, variance analysis; p, significance of the differences among the group investigated; n, number of cases; bw, body weight; MPA, medroxyprogesterone acetate.

three differently high doses of MPA. Reticular zone cell nuclei volume in the rats control group represented $95.61 \pm 1.03 \mu\text{m}^3$. After the application of 7.5 mg/kg bw of MPA the value of this parameter was $53.58 \pm 0.89 \mu\text{m}^3$; after the application of 15.0 mg/kg bw: $49.75 \pm 0.85 \mu\text{m}^3$; and after the application of 75.0 mg/kg bw: $47.68 \pm 0.92 \mu\text{m}^3$ ($F=600.53$; $p < 0.001$) (Table 3).

Percent representation of cortical proliferates and accessory adrenal glands was the following: their presence in the rats control group was evidenced in 58% of the animals; after the treatment with 7.5 and 15.0 mg/kg bw of MPA, they were present in 50%, and after the application of 75.0 mg/kg bw of MPA they were registered in 42% of the animals.

Tissue necrotic changes of the adrenal cortical gland were found in all groups of animals treated with MPA. In the frequency of this appearance there was observed difference depending on the doses used, i.e. the statistical analysis with χ^2 -test showed that this phenomenon had a statistical significance only after the

application of the highest doses of MPA ($p < 0.012$).

Gravimetry and morphologic analysis of the adrenal cortex, after application of MPA, demonstrated significant decrease of the adrenal gland weight and vol-

Table 4: Survey of morphologic characteristics of adrenal cortex after MPA application.

Parameter	Change after the application of MPA
Adrenal gland weight	Significantly decreased
Adrenal gland volume	Significantly decreased
Medulla volume	Non-significantly changed
Adrenal cortex volume	Significantly changed
Adrenal cortex thickness	Significantly decreased
Glomerular zone thickness	7.5 mg -no significantly changes
	15.0 mg- significantly changed
	75.0 mg- significantly decreased
Fascicular zone thickness	Significantly decreased
Reticular zone thickness	Significantly decreased
Glomerular zone cell nuclei volume	Significantly decreased
Fascicular zone cell nuclei volume	Significantly decreased
Reticular zone cell nuclei volume	Significantly decreased
Percent distribution of cortical proliferates and accessory adrenal glands	Co- in 58% of the animals
	7.5- in 50% of the animals
	15.0%-in 50% of the animals
Necrotic changes in adrenal cortex	75.0- in 42% of the animals
	The appearance has statistical significance after the application of 75.0 mg/kg bw of MPA

ume which was generally due to the reduction of its cortex. Also, it could be observed that the decrease of the cortical thickness was a result of the reduction of cell potential in all three cortical zones. Fascicular and reticular zones remarkably reduced after the application of three different MPA doses, while the glomerular zone thickness decreased when 15.0 and 75.0 mg/kg bw doses of MPA were applied (Table 4).

Discussion

Some authors considered that MPA did not involve in aldosterone secretion [20], still our investigation demonstrated that the long-term application of 15.0 and 75.0 mg/kg bw of MPA caused significant decrease of the glomerular zone cell potentials. This fact suggests that the application of high doses of MPA has a suppressive effect on glomerular zone. This phenomenon most likely has been due to the MPA capability to cause a complete suppression of ACTH secretion. Although the mineralocorticoid secretion from glomerular zone does not exclusively depend on the ACTH presence, still minimal ACTH quantities are necessary for normal functioning of this zone. This means that its total deficit could

cause mineralocorticoid secretion suppression which morphologic equivalent is the glomerular zone cell atrophy.

Cell potential decrease in fascicular zone, on the other hand, is an evidence for the existence of the MPA glyocorticoid feature and represents a morphologic index proved by numerous findings for decrease of the serum cortisol and ACTH in patients being administered MPA [8, 9, 21]. Many authors considered that MPA expresses its glyocorticoid effect through the biofeedback mechanism on hypothalamic-pituitary-adrenal axis [13]. In that way, the most remarkable was the adrenocortical component suppression [2]. It is characteristic that none of the patients being on MPA therapy showed signs of adrenal insufficiency even beyond the very low or equal to zero serum cortisol concentration [8, 9]. This means that MPA efficiently replaced the cortisol i.e. it expressed glyocorticoid characteristics. While, if it is given in very high doses, it could bring to clinical signs for hypercorticism. These facts suggest that MPA is a good replacement therapy for glyocorticoid hormones from the adrenal cortical gland.

On the other hand, decrease of the reticular zone cell potential represents a morphologic index for impairment of steroidogenesis in this zone. The mechanism of the steroidogenesis suppression could have contained more components. One of the main reasons for this phenomenon has been probably the ACTH quantity decrease from pituitary due to the possible MPA glyocorticoid action. Because secretion from reticular zone is controlled by this hormone, when it is lacking, atrophy in this zone probably ensues. Also MPA could have had a direct effect on the steroidogenesis itself, without decrease of the circulating gonadotrophins, through the inhibition of several enzymes necessary for normal steroidogenesis: 17-alpha-hydroxylase, 17,20-liase and 3-beta- and 17-beta-hydroxysteroid dehydrogenase [22]. Involvement of MPA in steroidogenesis could have run through steroid receptors as well. Binding of MPA for progesterone, androgenic and glucocorticoid receptors proved the direct MPA action on steroidogenesis [23].

In summary, quantitative analysis of histochemical changes in the adrenal cortical gland after the application of MPA demonstrated significant decrease of the adrenal cortex, i.e. decrease of the glomerular, fascicular and reticular zone thickness and significant decrease of the adrenocortical nuclei volume, changes which suggest that MPA causes adrenal cortical atrophy.

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