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ЕКСПЕРИМЕНТАЛЬНІ ДОСЛІДЖЕННЯ

THE IMPACT OF CHOLECALCIFEROL ON THE SEX HORMONES LEVEL AND SERUM BIOCHEMICAL INDICES IN RAT MALES WITH EXPERIMENTAL GONADOPATHY*

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The infertility is not only serious health care system's problem, but global social too, which disturbs millions of couples in the world. There are nearly 15% infertile couples in Europe now, but 20% in Ukraine [1], and due to war conveniences, this percentage may be growing. The male reproductive disturbances such as testicular deficiency, cryptorchidism, bad sperm quality, varicocele, erectile or ejaculation dysfunction, endocrine pathologies, congenital defects and ecological factors lead to infertility in 40–50% of cases. The male infertility is idiopathic in 50% of cases [2]. The testicles diseases cause the fertility declining in most cases [3] which determines the importance of studying of treatment methods of gonadopathies.

In recent times, vitamin D is noted among remedies suitable for effective correction of reproductive system. It is necessary to point out, that expression of vitamin D receptors is determined in the cells of reproductive systems' organs: testicles, prostate gland, seminal vesicles [4, 5]. The correlation between low vitamin D level and declining of motile and morphologically immature sperm cells was also detected. [6]. Vitamin D is considered to be essential for the adequate production of sex hormones, especially, testosterone (Ts) the lack of which leads to the disturbances of reproductive system in men [7].

The Ts level is higher in men without vitamin D deficiency in comparison with those who have D-hypovitaminosis. The decreasing of Ts/

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E_2 ratio has been determined in men with vitamin D deficiency [7].

There are a lot of reports about antioxidative activity of vitamin D and its derivates which allows to inhibit the inflammation and facilitates the reproductive function recovery as auxiliary methods.

Cholecalciferol inhibits the excessive inflammatory biomarkers and also regulates the expression of reproductive genes and androgens synthesis in testicles [8].

The literature data about vitamin D positive antioxidative effects indicate the new ways of reproductive disorders management and are considered to be the background of using of vitamin D for development of new medicines and schemes of treatment of different reproductive disturbances.

At the same time, despite great achievements of modern medicine and pharmacology, the problem of men' reproductive health is not completely resolved [9]. Data gained during last decades confirm the traditional methods of men hypofertility treatment are not effective enough. The using of vitamin D separately as well as in the complex schemes is a very promising direction in treatment of gonadopathies and reproductive system disturbances conditioned by them in men as well as in animal males.

Listed above defines the ***aim*** of this work: to determine the sex hormones levels and biochemical indices in serum of rat males with experimental testicles damage under the condition of vitamin D_3 correction used separately or in combination with Tribestan®.

MATERIALS AND METHODS

The experiment has been carried out on the sexually active 7-mo-old Wistar rat males, 250–300 g (n = 42). The investigation has been fulfilled according to the «National General Principles for Animal Researches Ethics» (Ukraine, 2001) which corresponds to the «European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes» (Strasburg, 1985). The rats have been kept under standard conditions of vivarium of SI «V. Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine», natural light sources and daily nutrition recommended for these animals, and water regime *ad libitum*.

The serotonin induced damage of spermatogenesis has been used to be the experimental model of reproductive function pathology. Experimental rats have been divided into groups: S-model group (animals with serotonin induced gonads, gonadopathy (GP)); S + solvent group (rats with experimental pathology which received seed oil — the solvent); S + vit. D_3 (animals with experimental pathology treated by vitamin D_3 *per os*); S + Tr (rats with GP which received Tribestan (Tr) — reference drug); S + vit. D_3 + Tr (animals with GP received *per os* Cholecalciferol and Tr). Intact males of the same age have been considered to be the control group.

The testicles pathology was induced by Serotonin hydrochloride (SHch) introduction during 14 days, subcutaneously at 3.3 mg/kg of body weight (ShanDong Octagon Chemicals Limited, China) [10, 11].

Reference drug Tr has been introduced once a day *per os* at 68 mg/kg of body weight before three days of SHch introduction, under the condition of SHch receiving (14 days) and during three days after last serotonin injection. During investigation the dose of medicine has been calculated using coefficient of species resilience on the base of human daily dose. The Cholecalciferol has been given according to the same scheme, in the volume of 0.5 ml contained 4000 IU (*per os*).

The solution of cholecalciferol was prepared on Apricot kernel oil from vitamin D_3 substance (powder, China, lot CHG20062009, which meet the requirements of quality standard GB 9840-2017).

The new pharmaceuticals dosage forms have been developed in-house based on well-known active pharmaceuticals ingredients and were produced in enough amount in the Technological department of Laboratory for analytical, physical and chemical investigations of SI «V. Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine».

After completing the course of substances administration, the animals have been removed from the experiment using quick decapitation, blood sampling has been fulfilled.

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) has been investigated by kinetic method using sets of reagents for AST and ALT determination in serum or in blood samples, AST SpL and ALT SpL accordingly (SpineLab).

The total metabolites of nitrogen oxide (NOx) cycle have been determined by Griess reaction [12], the content of Thiobarbituric acid reactive substances (TBARS) were measured using set of reagents («Agat», Ukraine), the concentration of arginine has been determined [13].

The serum levels of Ts and E_2 have been determined by ELISA («Chema», cat. # К209 and К208, Stat FAX 3200).

The statistical estimation of data obtained has been carried out using standard programs Microsoft Excel and Statistica 10.0. The Shapiro-Wilk test has been used to check if the data follows normal distribution. The probability of differences of means has been determined by Student t-test. Differences have been considered to be significant at $p < 0.05$

RESULTS AND THEIR DISCUSSION

During studying of Tr and vitamin D_3 influence the NOx concentration, the signs of oxidative stress (OS) were detected in all experimental groups of animals. The content of NOx in the S-model group exceeded by 2.5 times physiological normal concentration of these substances in control group ($p < 0.05$) (Table 1).

The NOx level was higher by 2.2 times in males of S + solvent group comparing with intact animals (Control group) (see Table 1; $p < 0.05$). It confirms the solvent does not influence the NOx production in experimental animals and demonstrates the role of nitrogen stress in the mechanisms of forming of this experimental pathology. The using of vitamin D_3 for gonadopathy correction in S + vit. D_3 group has caused the NOx decreasing by 31% relative to S-model group, however, haven't reached the indices of intact animals and were higher by 72% than last ones (see Table 1; $p < 0.05$). The correction of experimental gonadopathy by Tr has also led to OS declining. The NOx level was less by 37% in S + Tr group relative to S-model group (see Table 1; $p < 0.05$), but physiological normal indices of intact animals haven't been also reached in S + Tr group and their indices were higher by 56% (see Table 1; $p < 0.05$). The using of Tr and vitamin D_3 in complex was demonstrated positive effect as for OS defense. The concentration of NOx in S + vit. D_3 + Tr group was less by 56% relative to S-model group, but remained higher by 39% than the same indices in control group of animals (see Table 1; $p < 0.05$).

Further, the efficacy of D_3 and Tribestan administration on the free arginine concentration in animals with experimental gonadopathy has been studied. As it is known, the lack of amino acid arginine may indicate the developing of free radical processes under the condi-

Table 1

The content of nitrogen oxide cycle' metabolites, free arginine and thiobarbituric acid reactive substances in blood serum of rats, $\bar{X} \pm S_{\bar{X}}$ (n = 6)

Group	Indices		
	NOx, μmol/l	Arginine, μmol/l	TBARS, μmol/l
Control (intact animals)	0.88 ± 0.07	400.7 ± 8.1	1.51 ± 0.0
S-model	2.18 ± 0.12[1]	110.2 ± 4.8[1]	5.8 ± 0.2[1]
S + solvent	1.94 ± 0.15[1]	107.1 ± 2.0[1]	5.4 ± 0.3[1]
S + vit. D_3	1.51 ± 0.07[1,2]	130.2 ± 7.0[1]	3.6 ± 0.1[1,2]
S + Tr	1.37 ± 0.08[1,2]	215.2 ± 6.0[1,2,3]	3.0 ± 0.1[1,2,3]
S + vit. D_3 + Tr	1.22 ± 0.04[1,2,3]	240.1 ± 15.2[1,2,3]	2.6 ± 0.1[1,2,3]

Notes:

[1] Statistically significant difference vs. Control group ($p < 0.05$);

[2] Statistically significant difference vs. S-model group ($p < 0.05$);

[3] Statistically significant difference vs. S + vit. D_3 group ($p < 0.05$).

tion of oxidative stress (OS), because the one of its significant functions is to provide for a balance between pro- and antioxidative processes in tissues [14].

During investigation the deficiency of free arginine in blood serum has been detected in all experimental groups relative to Control (see Table 1). Arginine level was decreased almost by four times in animals of S-model group (see Table 1; $p < 0.05$). This enormous deficiency of amino acid was also detected in S + solvent group; the concentration of arginine was less almost by four times in this group relative to intact animals (see Table 1; $p < 0.05$) That indicates the absence of solvent influence on the oxidative — pro-oxidative processes.

The lack of arginine has been detected in S+vit. D_3 group; its level was three times less than in animals without gonadopathy (see Table 1, $p < 0.05$). Thus, the administration of vitamin D_3 to animals with GP hasn't considerably influenced free arginine level.

The treatment of testicles pathology by Tr has positively influenced the content of free arginine. The increasing of this amino acid concentration was at 95% in males of S + Tr group relatively to animals of S-model group. However, the physiological norm of intact animals hasn't been reached in S + Tr group and deficiency of arginine was 48% (see Table 1, $p < 0.05$).

The complex use of Tr and D_3 for S-GP correction has positive effect on free arginine level S + vit. D_3 + Tr. The concentration of this amino acid was by 2.2 times higher than in animals of S group (see Table 1, $p < 0.05$). Although, arginine concentration in this group hasn't reached physiological norm too and its deficiency was 40% (see Table 1, $p < 0.05$).

Therefore, the separate application of Tr and complex using of Tr and cholecalciferol were effective for restoration the pro-oxidative/antioxidative balance under the condition of experimental gonadopathy.

Free radical oxidation of lipids plays the key role in pathogenesis of diseases of men' reproductive system [15]. The increasing of lipid peroxidation (LP) leads to wasting of antioxidants of biological origin which is accompanied by decreasing of ability to regulate LP and contribute to LP products accumulation. During Tr and vitamin D_3 investigation the intensity of LP (TBARS) has been determined. TBARS are the secondary LP products which synthesized in the reaction with 2-thiobarbituric acid and indicate the degree of tissues damage.

Our investigation shows in animals with experimental testicles pathology the concentration of TBARS were almost four times higher than in control intact males (see Table 1, $p < 0.05$). This fact indicates the increasing of LP and OS development under the condition of SHch-induced model of GP. It is reported about LP increasing under the conditions of other models of gonadopathies [16].

In addition, LP increasing is confirmed by high concentration of NOx (nitrogen stress development) and decreased levels of free arginine (lack of antioxidants) in group of animals with experimental GP (see Table 1).

The content of TBARS was by 3.5 times higher in S + solvent group relative to intact males, which confirms weak capacity of solvent to influence the OS and LP development (see Table 1, $p < 0.05$). There were no differences between S-model and S + solvent groups.

The application of vitamin D_3 has decreased LP intensity and caused TBARS concentration lowering. The TBARS decreasing was by 38% less in S + vit. D_3 group relative to untreated animals with pathology (see Table 1, $p < 0.05$). Although, the content of TBARS was higher by 2.4 times, the indices of the intact animals haven't been reached (see Table 1, $p < 0.05$). As well known, vitamin D_3 is the strong antioxidant which may inhibit LP in tissues. Vitamin D_3 protects cells membranes from destroying by free radicals, supports their antioxidant capacity (considerably decreases iNOS expression, ROS and malondialdehyde production) [17, 18]. In this way vitamin D_3 inhibits LP and reduces OS.

The application of Tr as well as cholecalciferol for testicles damage correction has caused OS and LP reducing. The TBARS concentration was less by 1.9 times in S+Tr group comparing with GP group. However, the concentration of TBARS in S + Tr group hasn't reached intact animals' indices which were higher by two times (see Table 1; $p < 0.05$).

The complex application of Tr and vitamin D_3 was effective for reducing of LP intensi-

ty. Concentration of TBARS in S + vit. D_3 + Tr group has decreased by 2.2 times relative to S-model group of animals. However, TBARS concentration was also higher by 30% comparing with data obtained from intact animals (see Table 1; $p < 0.05$).

The using of Tr and vitamin D_3 in complex for GP treatment has demonstrated better effects, than single application of cholecalciferol as for protection against OS and LP reducing. Complex therapy was effective at the level of reference medicine.

According to J. M. Strickland (2021) data, cholecalciferol increases antioxidative capacity [20] which may contribute to fertility recovering, because from 25% to 87% cases of infertility are developed due to OS in men. It is considered oral supplements based on antioxidants may improve the quality of sperm decreasing oxidative damage [19].

Taking into account an important role of liver in the sex hormone and vitamin D metabolism, the studying of ALT and AST activity in blood serum of rats with GP and after treatment has been carried out.

As it is shown in Table 2, serotonin damage has caused the increasing of ALT by 2.4 times and AST — by 2 times comparing with control ($p < 0.05$).

Oral application of cholecalciferol or Tr as a monotherapy (groups S + Tr and S + vit.D_3) or their combined use haven't changed ALT activity which was statistically higher than in intact animals (see Table 2).

Meanwhile, the AST activity has increased in animals with GP, but has decreased after experimental correction of consequences of gonadopathy. Thus, the application of vitamin D_3 to be a monotherapy, has decreased AST concentration by 32% ($p < 0.05$) in compare with

Table 2

Activity of aspartate aminotransferase and alanine aminotransferase in blood serum of rat males of experimental groups $\bar{X} \pm S_{\bar{x}}$

Group	Index	
	ALT, U/L	AST, U/L
Control, n = 5	119.5 ± 15.3	137.0 ± 17.0
S-model, n = 5	272.5 ± 34.8[1]	278.9 ± 29.1[1]
S + solvent, n = 6	225.1 ± 44.3[1]	244.0 ± 17.5[1]
S + vit. D_3, n = 7	220.7 ± 23.4[1]	202.8 ± 11.6[1,2]
S + Tr, n = 8	244.5 ± 24.1[1]	212.0 ± 15.4[1,2]
S + vit. D_3 + Tr, n = 8	203.0 ± 19.7[1]	202.4 ± 11.8[1,2]

Notes:
[1] Statistically significant difference vs. Control group ($p < 0.05$);
[2] Statistically significant difference vs. S-model group ($p < 0.05$).

Table 3

The sex hormones concentration in blood serum of the experimental animals, $\bar{X} \pm S_{\bar{x}}$

Group (n = 5–6)	Total Testosterone, nmol/l	Estradiol, pmol/l	Ts/E_2, $\times 10^{-3}$
Control (intact animals)	20.5 ± 1.6	31.0 ± 2.2	0.57 ± 0.05
S-model	7.3 ± 0.2[1]	30.4 ± 1.9	0.25 ± 0.02[1]
S + solvent	7.0 ± 1.8[1]	30.9 ± 4.1	0.26 ± 0.02[1]
S + vit. D_3	8.5 ± 0.7[1]	28.3 ± 1.0	0.31 ± 0.02[1]
S + Tr	14.2 ± 0.6[1,2]	28.8 ± 1.3	0.50 ± 0.03[2]
S + vit. D_3 + Tr	15.3 ± 0.4[1,2]	28.7 ± 2.0	0.56 ± 0.04[2]

Notes:
[1] Statistically significant difference vs. Control group ($p < 0.05$);
[2] Statistically significant difference vs. S-model group ($p < 0.05$).

S-model group of animals. The application of complex therapy consisted from vitamin D_3 and medicine of base therapeutic group (group of animals S + vit.D_3 + Tr) the AST concentration in males' blood serum has decreased by 32% too ($p < 0.05$) comparing with untreated animals. Therefore, vitamin D as a monotherapy, as well as in combination with Tr, has positively influenced the AST level.

It is reported that exogenous application of arginine has normalized liver transaminases concentration in infertile men which associated with decreasing of sex steroid-binding globulin and increasing of free androgen index and testosterone-estradiol ratio [20]. During our investigation the endogenic increasing of this amino acid (groups S + Tr and S + vit. D_3 + Tr, see Table 2) has been detected. This is what explains the influence of experimental correction on the AST level.

During investigation of Tr and vitamin D_3 impact on the sex hormones levels and after determination of androgen/estrogen status of experimental animals, it has been shown that SHch-induced gonadopathy has caused the deficiency of total Ts in serum of animals of all groups (Table 3).

The deficiency of Ts was almost by three times less in S-model group relative to intact males (see Table 3; $p < 0.05$). The Ts declining after application of SHch has confirmed the androgen saturation of organism was decreased under the condition of this experimental model [21]. The same (three times) shortage of Ts has been detected in (S + solvent) group which indicated the solvent' androgenic activity absence (see Table 3; $p < 0.05$).

The application of vitamin D_3 for GP treatment hasn't demonstrated even small androgen corrective influence. The concentration of Ts in S + vit. D_3 group has been detected at S+model level (see Table 3; $p > 0.1$).

The using of Tr for GP correction has considerably influenced the androgen synthetized function. The Ts concentration was detected by 95% higher in S + Tr rats than in S+model group of animals (see Table 3; $p < 0.05$). However, the concentration of Testosterone in this group has remained almost by 31% lower than intact animal level (see Table 3; $p < 0.05$). Other literary sources report the same increasing of Ts in rats with serotonin induced GP [21].

The complex application of both studied components has demonstrated positive effect on the androgen synthetizing function too. The concentration of Ts in S + vit. D_3 + Tr group was by 2.1 times higher than in animals of S + model group (see Table 3; $p < 0.05$). Although, Ts concentration values in this group haven't reached values of animals from Control group and were by 25% lower than their hormone levels (see Table 3; $p < 0.05$).

It is necessary to note, during investigation, E_2 concentration in all experimental groups was detected to be corresponded to the values of rats of intact group (see Table 3). This fact confirms the absence of effect of the experimental model as well as used medicines on the estradiol synthetizing activity in males with serotonin-induced testicles damage.

Ts/E_2 ratio is the marker of organism androgenization (estrogenisation). The calculation has shown Ts/E_2 ratio has been declined by 2.3; 2.1 and 1.8 times in experimental rats of S-model, S+solvent, S + vit. D_3 groups accordingly in comparison with intact animals (see Table 3; $p < 0.05$). Taking into consideration E_2 concentration was at normal level in rats of all these groups, the decreasing of Ts/E_2 ratio has occurred due to deficiency of androgens.

The Ts/E_2 ratio was at level of intact animals in S + Tr and S + vit. D_3 + Tr groups which have been treated by reference medicine to be a monotherapy and by its combination with vitamin D_3 (see Table 3). The Ts/E_2 ratio has been two times higher in S + Tr and S + vit. D_3 + Tr groups in comparison with indices of S-model group of rats (see Table 3; $p < 0.05$). It can be noted, the application of this correction has demonstrated positive effect as for SHch-induced damage, because E_2 concentration was at level of indices of intact males, Ts content almost reached physiological values and wasn't very low.

As shown earlier [22], the application of cholecalciferol in complex with Tr has recovered sexual behaviour, reproductive function and, it is especially important, the development of offspring of rat males with experimental SHch-induced damage of testicles. The complex treatment by cholecalciferol and Tr has

improved not only researched biochemical indices. Therefore, it is reasonable to administrate vitamin D in complex schemes for infertility treatment and may be used for development of new treatment methods of men' reproductive disorders of different etiology.

CONCLUSIONS

1. Serotonin-induced experimental modelling of testicles hypofunction causes the pathological changes of testicular tissue in rats which manifest in biochemical indices of blood serum. The increasing of metabolites of nitrogen oxide cycle, free arginine and TBARS have been detected under the condition of gonadopathy in rats. Absolute and relative hypotestosteronemia, the activation of alanine aminotransferase and aspartate aminotransferase has been determined.
2. The application of cholecalciferol has considerably decreased NOx and TBARS concentrations, but hasn't affected on arginine level which has declined with the gonadopathy. Vitamin D has caused reducing of aspartate aminotransferase activity and hasn't had androgenic and estrogenic effects.
3. The application of Tribestan has led to the pro-oxidative/antioxidative balance recovery under the condition of experimental gonadopathy. This medicine has reduced the high level of aspartate aminotransferase activity, increased testosterone level and normalized testosterone/estradiol ratio in blood serum of experimental animals.
4. Under the condition of experimental serotonin-induced gonadopathy the application of vitamin D and Tribestan in complex has led to improvement of androgenic status and has demonstrated better effect as for protection against oxidative stress and lipid peroxidation reducing than cholecalciferol as a monotherapy.

REFERENCES

1. Khmil SV, Mayorova OY, Dudchuk IV. *Aktual'ni pytannja pediatrii', akusherstva ta ginekologii'* 2020;2: 91–97. https://doi.org/10.11603/24116-4944.2019.2.10819
2. Kumar N, Singh AK. *J Hum Reprod Sci* 2015;8(4): 191-196. https://doi.org/10.4103/0974-1208.170370
3. Sharma A. *Ann Clin Lab Res* 2017;5(3): 188. https://doi.org/10.21767/2386-5180.1000188
4. Angelis C, Galdiero M, Pivonello C, et al. *Rev Endocr Metab Disord* 2017;18(3): 285-305. https://doi.org/10.10007/S 11754-017-9428-0.
5. Boisen IM, Hansen LB, Mortensen LJ, et al. *J Steroid Biochem Mol Biol* 2017;173: 215-222. https://doi.org/10.1016/j.jsbmb.2016.09.023.
6. Karras S, Anagnostis P, Kotsa K, Goulis DG. *Andrology* 2016;4(3): 542-544. doi: 10.1111/andr.12173
7. Blomberg J, Gerner LG, Andersson AV, et al. *Human Reprod* 2016;31(8): 1875-1885. doi:10.1093/humzep/dew 1952.
8. Ding C, Wang Q, Hao Y, et al. *Biochem Biophys Res Commun* 2016;473(1): 161-167. https://doi.org/10.1016/j.bbrc.2016.03.072.
9. Marcelli M, Mediwala SN. *J Investig Med* 2020;68(2): 335-356. https://doi.org/10.1136/jim-2019-001233
10. Zajchenko GV, Jakovleva LV, Butenko IG, Larjanovs'ka JuB. *Farmakologija ta likars'ka toksykologija* 2008;1(3): 74-79.
11. Smolienko NP, Korenieva YeM., Chystiakova EYe, et al. *Problems of Endocrine Pathology* 2022;79(3): 72-80. https://doi.org/10.21856/j-PEP.2022.3.10
12. Patent 31600 Sposib kil'kisnogo viznachennja nitrit-anionu v biologichnij ridini.
13. Mehed OB, Jakovenko BV, Tretjak OP. Bioorganichna himija: Navchal'nij posibnik, *Chernigiv,* 2013: 208 p.
14. Gorpinchenko II, Stus' VP, Malyshkin DI, Polion NJu. Muzhskoe besplodie: jetiologija, patogenez, klassifikacija, diagnostika i metody lechenija: Monografija, *Dnepr,* 2016: 344 p.
15. Vaos G, Zavras N. *World Journal of Methodology* 2017; 7(2): 37 https://doi.org/10.5662/wjm.v7.i2.37
16. Bedir F, Kocaturk H, Ozgeris FB, et al. *Revista Internacional de Andrología* 2022;20(1): 17-23. https://doi.org/10.1016/j.androl.2020.05.008
17. Strickland JM, Wisnieski L, Mavangira V, Sordillo LM. *Antioxidants (Basel)* 2021;10(9): 1420-1437. https://doi.org/10.3390/antiox10091420
18. Leal LM, Lima LA, de Aquino PA, et al. *Eur J Pharmacol* 2020;879: 173099.
19. de Ligny W, Smits RM, Mackenzie-Proctor R, et al. *Cochrane Database Syst Rev* 2022;5(5): CD007411. https://doi.org/10.1002/14651858.CD007411.pub5
20. Bondarenko VO, Skornjakov ЄI, Alekseeva II, et al. *Problemi endokrinnoї patologiї* 2013;2: 56-61.

21. Brechka NM, Sirotenko LA, Koreneva EM, et al. *Ukraïns'kij zhurnal medicini, biologiï ta sportu* 2020;5(2): 172-177. https://doi.org/10.26693/jmbs05.02.172

22. Smolienko N, Korenyeva YM, Marakhovskiy I, et al. *Bulletin of Problems Biology and Medicine* 2022;1(2): 240. https://doi.org/10.29254/2077-4214-2022-2-1-164-240-248

THE IMPACT OF CHOLECALCIFEROL ON THE SEX HORMONES LEVEL AND SERUM BIOCHEMICAL INDICES IN RAT MALES WITH EXPERIMENTAL GONADOPATHY

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The testicular deficiency, cryptorchidism, varicocele, endocrine pathologies are the main causes of men infertility. The internal diseases of testicles lead to fertility declining in the major part of cases which explains the importance of studying of gonadopathies' methods of correction. In recent times, vitamin D is noted among remedies suitable for effective correction of reproductive system.

The aim of investigation: to determine the sex hormones levels and biochemical indices in serum of rats' males with experimental testicles damage under the condition of vitamin D_3 correction used separately or in combination with Tribestan®.

Materials and methods. The experiment has been carried out on mature Vistar rats' males; group of animals: S-model (animals with serotonin-induced gonads damage, gonadopathy (GP)); S + solvent (rats with gonadopathy which received solvent); S + vit. D_3 (animals with gonadopathy received vitamin D_3); S + Tr (rats with gonadopathy obtained Tribestan (Tr) — reference medicine); S + vit. D_3 + Tr (animals received both remedies); control — intact males. The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), general content of nitrogen oxide cycle metabolites (NOx), concentration of TBARS and sex hormones Ts and E_2 have been determined; their ratio has been calculated.

Results. The NOx was increased in S-model rats. The application of vit. D_3 or Tr has caused decreasing of NOx concentration in comparison with S-model group. The complex using of vit. D_3 and Tr has had positive effect as for protection against oxidative stress (OS). The concentration of NOx in rats of S + vit. D_3 + Tr was by 56% less than in S-model group, but, as in the other experimental groups of animals, has remained increased relative to control group.

The deficiency of free arginine in blood serum has been detected in all experimental groups comparing with Control group. The application of vit. D_3 hasn't affected on the arginine content. Tr has positively influenced the arginine level: the increasing of concentration of this amino acid was 95% relative to S-model group, but was less by 48% than values of intact animals. After application of Tr and vit. D_3 together the concentration of arginine was higher by 2.2 times than in rats of S-model group, but hasn't reached the values of intact animals.

The concentration of TBARS has increased under the condition of GP. The using of vit. D_3 has led to reducing, but not to normalizing of TBARS content. The concentration of TBARS in rats of S + Tr group was by 1.9 times lower than in rats with GP and by two times higher than in intact animals. Simultaneous using of Tr and vit. D_3 has been effective as for reducing of lipid peroxidation (LP) intensity. The concentration of TBARS in S + vit. D_3 + Tr group has declined by 2.2 times comparing with values of animals with GP. The combination of Tr and vitamin D_3 was more effective than cholecalciferol single using as for protection against OS and LP reducing and has demonstrated activity at the level of reference drug.

GP has led to increasing of ALT activity 2.4 times and AST — by 2 times comparing with control group. The application of Tr, vit. D_3 or their complex hasn't changed ALT activity which was statistically higher than intact animals' values. The activity of AST was increased in animals with GP and reduced after experimental correction of GP consequences. Vitamin D_3 monotherapy has reduced by 32% the activity of AST comparing with animals with GP. AST concentration in males' blood serum was decreased too with the combined use of Tr and vitamin D_3 comparing with untreated animals. Thus, vitamin D has positively impacted the AST level in combined use with Tr as well as monotherapy.

Serotonin-induced experimental GP has caused the declining of general Ts concentration in blood serum of animals of all experimental groups. The application of vitamin D_3 hasn't demonstrated androgen correcting activity. Tr has positively influenced androgen synthesized function: Ts concentration has increased by 95% comparing with S-model group, but remained lower by 31% than in intact animals. The Ts concentration in S + vit. D_3 + Tr group was 2.1 times higher than in animals with GP. However, Ts values in this group haven't reached Control group values and were by 25% less. The concentration of estradiol in all experimental groups has corresponded to the values of intact animals. The calculation of Ts/E_2 ratio has shown to be decreased in S-model, S + solvent, S + vit. D_3 groups in comparison with intact animals. The Ts/E_2 ratio has normalized in groups with GP treated by reference drug separately or in combination with vitamin D_3.

Conclusions: The application of cholecalciferol has considerably decreased NOx and TBARS concentrations, but hasn't affected on arginine level which has declined with the gonadopathy. Vitamin D has caused reducing of aspartate aminotransferase activity in serum and hasn't had androgenic and estrogenic effects. The application of Tribestan has led to the pro-oxidative/antioxidative balance recovery under the condition of experimental gonadopathy. This medicine has reduced the high level of aspartate aminotransferase activity, increased testosterone level and normalized testosterone/estradiol ratio in blood serum of experimental animals. The application of vitamin D and Tribestan in combination has led to improvement of androgenic status and has demonstrated better effect as for protection against oxidative stress and lipid peroxidation than cholecalciferol as a monotherapy.

Keywords: vitamin D, gonadopathies, estradiol, biochemical indices, testosterone, cholecalciferol.

ВПЛИВ ХОЛЕКАЛЬЦИФЕРОЛУ НА РІВЕНЬ СТАТЕВИХ ГОРМОНІВ ТА БІОХІМІЧНІ ПОКАЗНИКИ СИРОВАТКИ КРОВІ САМЦІВ ЩУРІВ ІЗ ЕКСПЕРИМЕНТАЛЬНОЮ ГОНАДОПАТІЄЮ

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Основними чинниками чоловічого безпліддя є тестикулярна недостатність, крипторхізм, варикоцеле, ендокринні розлади тощо. У більшості випадків зниження фертильності викликано внутрішнім захворюванням яєчок, що обумовлює важливість вивчення методів корекції гонадопатій. Останнім часом серед засобів ефективного впливу на репродуктивну систему виділяють вітамін D.

Мета дослідження: визначення рівня статевих гормонів та біохімічних показників сироватки крові самців щурів із експериментальним ураженням сім'яників за умов корекції вітаміном D_3, застосованого самостійно або у комбінації з Трібестаном.

Матеріали та методи. Дослідження виконано на дорослих самцях щурів популяції Вістар; групи тварин: S-модель (тварини з експериментальною моделлю серотонінового ураження гонад, гонадопатія (ГП)); S + розчинник (щури, які на тлі гонадопатії отримували розчинник; S + віт. D_3 (тварини на тлі ураження сім'яників отримували вітамін D_3); S + Тр (щури з гонадопатією отримували Трібестан (Тр) — референтний препарат); S + віт. D_3 + Тр (тварини одержували обидва препарати); контроль — інтактні самці. Визначали активність аспартатамінотрансферази (АСТ) та аланінамінотрансферази (АЛТ), загальний вміст стабільних метаболітів циклу оксиду азоту (NOx), вміст ТБК-активних продуктів; рівні статевих гормонів Тс та E_2, розраховували їх співвідношення.

Результати. У щурів групи S-модель підвищувалися NOx. Застосування віт. D_3 або Тр призвело до зниження вмісту NOx відносно групи S-модель. Комплексне застосування віт. D_3 та Тр мало позитивний ефект щодо захисту від оксидативного стресу (ОС). Концентрація NOx у групі (S + віт. D_3 + Тр) була на 56% нижчою відносно значень у групі S-модель, але, як і в інших групах, залишалася підвищеною відносно контрольних тварин.

Було виявлено дефіцит вільного аргініну в сироватці крові в усіх піддослідних групах відносно Контролю. Застосування віт. D_3 не впливало на рівень аргініну. Тр позитивно діяв на вміст аргініну: підвищення концентрації амінокислоти становило 95% по відношенню до групи S, але було нижче значень інтактних тварин на 48%. При застосуванні Тр та віт. D_3 разом концентрація аргініну була вищою в 2,2 рази за групу S, але даних інтактних тварин не досягала.

При ГП вміст ТБК-активних продуктів підвищувався. Застосування віт. D_3 призводило до їх зниження але не нормалізувало. Концентрація ТБК-активних продуктів у тварин групи S + Тр була нижче відносно групи із ГП у 1,9 рази та вище за інтактних у два рази. Комбіноване використання Тр та віт. D_3 було ефективне щодо зниження інтенсивності перекисного окиснення ліпідів (ПОЛ). Вміст ТБК-активних продуктів у групі S + віт. D_3 + Тр знижувався у 2,2 рази відносно значень у тварин із ГП. Комбінація Тр та віт. D_3 мала кращу дію, ніж використання одного холекальциферолу, щодо захисту від ОС та зниження ПОЛ та діяло на рівні референтного препарату.

ГП призводила до підвищення активності АЛТ в 2,4 рази та АСТ — в 2 рази порівняно з контролем. Застосування Тр, віт.D_3 або їх сумісне використання не змінювало активності АЛТ, яка була вірогідно вище за показники інтактних тварин. Активність АСТ підвищилась у тварин із ГП та знижувалась при експериментальній корекції наслідків ГП. Монотерапія віт. D_3 знижувала активність АСТ на 32% порівняно з групою тварин із ГП. При сумісному застосуванні препаратів АСТ у сироватці крові самців також зменшувалась порівняно з тваринами без корекції. Тож віт. D позитивно впливав на рівень АСТ при застосуванні як окремо, так і в комбінації із Тр.

Моделювання серотонінової ГП призводило до зниження рівня загального Тс у сироватці крові в усіх групах досліджуваних тварин. Застосування віт. D_3 для корекції ГП не мало андроген-коригуючого впливу. Тр сприяв значному позитивному впливу на андроген-синтетичну функцію: вміст Тс

став на 95% вищим за таки показники у групі S-модель, але нижче рівня інтактних тварин майже на 31%. В групі S + віт. D_3 + Тр концентрація Тс виявилася у 2,1 рази вищою за показники тварин із ГП. Але значення Тс і в цій групі не досягали значень тварин групи Контроль і були на 25% нижче за них. Рівень естрадіолу в усіх експериментальних групах відповідав значенням інтактних щурів. Розрахунок співвідношення Тс/E_2 показав, що у щурів експериментальних груп S-модель, S + розчинник, S + віт. D_3 відбулося зменшення Тс/E_2 по відношенню до інтактних тварин. В групах, де проводилося корегування ГП референтним препаратом самостійно та у комбінації з віт. D_3, співвідношення Тс/E_2 нормалізувалося.

Висновки: Використання вітаміну D_3 проявляє ефективну дію стосовно зниження рівня NOx та ТБК-активних продуктів, але не впливає на рівень аргініну, який знизився при гонадопатії; викликає зниження активності аспартатамінотрансферази у сироватці крові та не проявляє андрогенного та естрогенного ефекту. За умов гонадопатії застосування Трібестану призводить до відновлення прооксидантно/антиоксидантного балансу; знижує рівень активності ферменту аспартатамінотрансферази, підвищує рівень чоловічого статевого гормону та нормалізує співвідношення тестостерон/естрадіол у сироватці крові піддослідних щурів. Комбіноване використання вітаміну D_3 сумісно з Трібестаном покращує андрогенний статусу організму та має кращу дію, ніж використання одного холекальциферолу, щодо захисту від оксидативного стресу та зниження перекисного окислення ліпідів, діючи на рівні референтного препарату.

Ключові слова: вітамін D, гонадопатії, естрадіол, біохімічні показники, тестостерон, холекальциферол.