ISSN 2518-1432 (Online); ISSN 2227-4782 (Print) https://doi.org/10.21856/j-PEP.2022.2.12

THE INFLUENCE OF VITAMIN D3 USED INDEPENDENTLY, IN COMBINATION OR IN THE PHARMACEUTICAL COMPOSITION WITH PROSTATE CORRECTOR ON THE SEX HORMONES LEVELS IN RATS WITH EXPERIMENTAL PROSTATITIS*

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World Health Organization declares more than one billion people with vitamin D deficiency. Hypovitaminosis-D problem is actual in Ukraine, where vitamin D deficiency registered in 81,8 % of cases [1, 2].

Nowadays, the traditional view of the role of vitamin D in calcium and phosphate metabolism and its influence on the bones tissue is supplemented by new data about impact of vitamin D deficiency on the development of reproductive function disturbances. It has been determined that vitamin D is necessary for adequate steroid hormones production and full-fledged spermatogenesis, but its insufficiency causes the development of androgen deficiency and pathospermia, which generally leads to subfertility. It is reported the expression of vitamin D receptors in the cells of reproductive organs such as testicles (Sertoli cells, seminiferous tubules, spermatogonial stem cells and so on), in epididymis, seminal vesicles (SV), prostate gland (PG) and even in the sperm cells was determined [3].

It has been shown the using of vitamin D during one month has contributed to improvement of Leydig and Sertoli cells functioning, has dose-dependently increased the synthesis of testosterone (Ts) and activity of mitochon-

* The work was performed in the State Institution «V. Danilevsky Institute for Endocrine Pathology Problems of the National Academy of Medical Sciences of Ukraine» within the framework of the topic "Determination of vitamin D deficiency and insufficiency in the development of sex glands dysfunction, the grounding of treatment" (State registration number: 0119U102387).

The institution that finances the study is the National Academy of Medical Sciences of Ukraine.

The authors assume responsibility for the published work.

The authors guarantee absence of competing interests and their own financial interest when carrying out the research and writing the article.

The manuscript was received by the editorial staff 18.04.2022.

drial dehydrogenase in the Leydig cells' culture under the influence of luteinizing hormone. All of that indicate vitamin D necessity for adequate production of steroid hormones and its deficiency may negatively affects the male reproductive function [4, 5].

Ts, in turn, is the regulator of vitamin D receptors' activity in the testicles. The treatment with vitamin D has led to increasing of male sex hormone level in blood serum in patients with D-hypovitaminosis [6]. Moreover, the using of vitamin D in men with idiopathic oligoasthenozoospermia during three months, even not counting its blood concentration amid treatment, has improved sperm motility [7]. This suggests that using vitamin D for therapy of reproductive function disturbances in men with gonad and prostate gland pathologies is possible under the condition of D-hypovitaminosis as well as without it. However, the experimental grounding of vitamin D administration on the pathology models that simulate the most wide-spread variants of infertility is needed to confirm this. These pathologies include chronic inflammatory processes in PG and SV, because subfertility in men with prostatitis remains one of the main reasons of infertile marriages. Also, it is not known how the using of vitamin D simultaneously with the base therapy of nonbacterial prostatitis affects the indices of the incretory function of gonads. To date, there aren't experimental data about changes of hormone synthetic function of testicles when prostatitis modelling as well as of cholecalciferol (D_3) administrating together with prostate protectors. Therefore, carrying out researches on the reasonable using of vitamin D for correction of disturbances in the functioning of men's gonads is highly relevant.

Taking into consideration data listed above, the **aim** of this work was to research the using of vitamin D (as a monotherapy or in complex schemes) for correction of sex hormones levels and fructose concentration in rats' males with experimental prostatitis (EP).

MATERIALS AND METHODS

The investigations have been carried out on Vistar, sex active rats' males of 250–300 g body mass, which have been kept under standard conditions of vivarium, in the natural light and water regime *ad libitum* according to the national «Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes».

EP has been modelled using cryo traumatizing of ventral part of prostate gland with the help of WARTNER Cryo Freeze Wart & Verruca Remover® (Omega Pharma International, Belgium). [8]. Investigated compounds have been introduced from 15th day after manipulation during three weeks. Vitamin D has been used per os, in dose which corresponded to 4000 IU [9]. The introduction of referent prostate protector Prostatilen and new pharmaceutical composition of Prostatilen (Pr) and vitamin D has been fulfilled rectally, into high part of rectum, to a depth of 20–25 mm in the volume of 0,5 ml. The non-traumatic, semi-solid catheter with diameter of 3 mm has been used for this purpose. Catheter was made from standard intravenous catheter G23 Angelmed®.

The experiment has been carried out on such groups of animals: EP + solvent (oil), EP + Pr (rec), EP + D_3 (per os), EP + (Pr + D_3) (rec) group, EP + D_3 (per os) + Pr (rec) and Control group, which hasn't been operated. The vitamin D oil seed solution was used in the experiment. The 0,5 % Prostatilen rectal gel (prepared according to patent) was used as a drug of comparison [10].

Animals have been removed from the experiment on the 36th day after operation using quick decapitation. The SV masses and their fructose concentration have been determined [11]. Seminal fructose concentration is considered to be the indirect index of androgenization. The blood serum levels of estradiol (E_2) and general testosterone Ts have been determined using immunoassay sets («Khema» firm). The index of relative androgenization/estrogenization — Tc/E_2 has been calculated. Data obtained have been estimated using program Excel 2003. Under the condition of normal distribution data have been shown as arithmetic mean (x) and standard deviation ($\pm S_x$). Student's T-test has been used for estimation of probability of mean deviation [12]. Deviations have been considered to be significant at $P < 0,05$.

Table

The concentration of sex hormones in blood serum of rats, ($\bar{X} \pm S_{\bar{X}}$)

Group	General testosterone, nmol/l	Estradiol, nmol/l	Testosterone/ Estradiol, SU $\times 10^{-3}$
Control (false operated), n = 5	20,2 ± 1,2	0,03 ± 0,01	0,56 ± 0,06
EP+solvent, n = 5	8,2 ± 0,6 [1)]	0,04 ± 0,01	0,27 ± 0,04 [1)]
EP+Pr(rec), n = 5	17,6 ± 1,3 [2)]	0,04 ± 0,00	0,47 ± 0,04 [2)]
EP+D_3(per os), n = 6	10,2 ± 1,2 [1)]	0,04 ± 0,01	0,26 ± 0,03 [1)]
EP+Pr(rec)+D_3(per os), n = 6	19,1 ± 1,1 [2)]	0,04 ± 0,00	0,59 ± 0,08 [2)]
EP+(Pr+D_3)(rec), n = 6	15,3 ± 0,9 [1, 2)]	0,04 ± 0,01	0,39 ± 0,10 [2)]

Notes:

[1)] difference with Control group (false operated);

[2)] difference with group EP + solvent; $p < 0,05$.

RESULTS AND THEIR DISCUSSION

During investigation of influence of Pr and vitamin D on the sex hormones level in serum the deficiency of general Ts has been detected in such groups of animals as EP + solvent, EP + D_3, EP + (Pr+D_3)(rec) in contrast to Control groups (false operated) of males. The Ts level has decreased by 2,3 in EP+solvent group, in group of males EP + vit. D_3 (per os) — has been as many as twice and in EP + (Pr + D_3)(rec) group has declined 1,3 times lower than in control group of animals (table; $p < 0,05$).

Using of Pr as a monotherapy of EP has had positive effect on androgen-synthetic function of testicles. The Ts concentration in Ep + Pr group has almost reached the same levels of Control group of animals (see table) ($p < 0,05$). It must be noted that all of experimental groups when Pr was used ((Ep + Pr(rec)), EP + Pr(rec) + D_3(per os), EP + (Pr + D_3)(rec)) have demonstrated reliably highest Ts level comparing with groups when medicine hasn't been administrated (see table, $p < 0,05$). It can be explained by the fact that Pr is the peptide bioregulator (cytomedin), which is obtained from bull's prostate glands. The activity of these peptides is tissue-specific for organs from which they were extracted [13], in this case — prostate gland. It is well known that prostate tissues contain a lot of arginine and zinc, which directly influence the Ts synthesis.

Monotherapy by vitamin D_3 hadn't any effects as for improving of androgenic status in EP. The concentration of general Ts in EP + D_3(per os) group was at its level in Ep + solvent group, less by 50 % and 42 % comparing with Control (false operated) and EP + Pr(rec) accordingly (see table; $p < 0,05$). The biological effects of vitamin D_3 are fulfilled through the interaction with their specific receptors (vitamin D receptors or VDR) which are located in the nucleus of cell of reproductive organs including Leydig cells [14]. In the cells culture of human testicular tissue vitamin D leads to the increasing of Ts production [15], but literature data as for vitamin D impact on the testosterone blood level are controversial enough. There were some scientific researches that have demonstrated vitamin D_3 effects on the testosterone synthesis [16], although, other investigations haven't determined correlation between vitamin D_3 and Ts [17, 18]. That's why, this problem has to be further investigated.

Combined therapy, which used vitamin D_3 and Pr for EP treatment, has positively affected the androgen-synthetic function of testicles that has led to general Ts increasing in blood serum of experimental rats. Thus, the general Ts concentration in EP + Pr(rec) + D_3(per os) group was similar to indices of rats in Control group (see table; $p < 0,05$). At the same time, general Ts level in EP + (Pr + D_3)(rec), which has received pharmaceutical composition, has merely approximated to Control group indices and was less by 24 % (see table; $p < 0,05$). It means, using Pr rectally in combination with

vitamin D_3 oral administration have demonstrated better effect as for androgen status of experimental rats. Probably, it may be explained by particularities of vitamin's metabolism: vitamin D_3 (cholecalciferol) orally used is initially metabolized in liver and kidneys, when is transformed into active substance — calcitriol. Then, it binds with carrier protein and is transferred to target organs: intestinal tract, bones, kidneys etc. Cholecalciferol is absorbed in small intestine [19], but, under the condition of rectal use, such transformation doesn't happen or takes place very slowly which can influence its activity.

During investigation of another sex hormone — estradiol, it has been determined that its blood serum concentrations in all groups of experimental animals were at Control group level. This fact confirms the absence of influence of researched remedies (Pr and vitamin D_3) on the estradiol synthesis in rats with CP when monotherapy as well as combined scheme were used.

The well-known marker of organism androgenization (estrogenization) is Ts/E_2 ratio. It has been calculated that this index has been decreased nearly by 50 % and 46 % in EP + solvent and EP + D_3(per os) groups accordingly ($p < 0,05$; see table). Taking into account normal concentration of E_2 in those groups, the decreasing of Ts/E_2 ratio has occurred only due to Ts deficiency.

The Ts/E_2 ratio was at level of Control group ($p < 0,05$) in the experimental groups treated with Pr independently as well as in the combination with vitamin D_3 (per os or rec.). Ts/E_2 index in EP + Pr(rec), EP + Pr(rec) + D_3(per os) and EP + (Pr + D_3)(rec) was more twice than EP+solvent group. Because in these groups the concentrations of sex hormones were at control level, it can be noted chosen drug combinations have had positive effect as for EP treatment.

Among large number of substances identified in sperm fluid, fructose is becoming increasingly important. Fructose is produced and secreted by SV. Its synthesis is regulated by androgens and directly displays organism's androgen saturation and specific activity of men's sex hormone [20]. The main role of fructose — sperm cells activating as far as it is the basic sours of energy for them. I.e., fructose produced by SV, after penetration into seminal fluid it makes sperm cells motile. Therefore, during this researching the concentration of fructose has been detected in the experimental animals.

The investigation of fructose concentration in EP + solvent group has shown its level to be decreased nearly three times comparing with Control group (Fig. 1, $p < 0,05$). The calculation of fructose concentration in whole organ EP + solvent group has detected its three-time decreasing level too comparing with Control group (see Fig. 1; $p < 0,05$). At the same time, the SV masses in experimental males have kept at physiological levels in control rats (see Fig. 1; $p < 0,05$). As it is known, the decreasing of fructose concentration is the indicator of disturbance of secretory function of SV. The functioning of SV is depended from Ts supplement to the epithelial cells of this organ [21], that's because, hypofunctioning of this accessorial gland can be connected with declining of androgen saturation (hypotestosteronemia) due to experimental conditions.

The treatment of EP with prostate corrector Pr (EP + Pr(rec) group) has facilitated the normalization of fructose concentration. As for (EP + Pr(rec) group, the fructose concentration in these males has been twice decreased, (see Fig. 1, $p < 0,05$). The calculation of fructose concentration in whole organ has shown some improvement of this index: it was 72 % more than EP + solvent group, but 46 % less than control animals. Thus, SV masses in (EP + Pr (rec) group were within normal parameters of control group of animals (see Fig. 1; $p < 0,05$). The using of prostate protector Pr has led to increasing of Ts level in (EP + Pr(rec) group of males, that's why, it could improve functional activity of SV and increase fructose level.

The monotherapy with vitamin D_3 has led to fructose level increasing by 50 % in males of EP + D_3(per os) group comparing with EP + solvent rats (see Fig. 1; $p < 0,05$), however, this group's parameters haven't reached Control group and were nearly twice less (see Fig. 1; $p < 0,05$). However, the concentration of fructose of whole organ in group EP + D_3(per os) was equal to Control group parameters and was three-time more than in group with experimental pathology (EP + solvent). It is im-

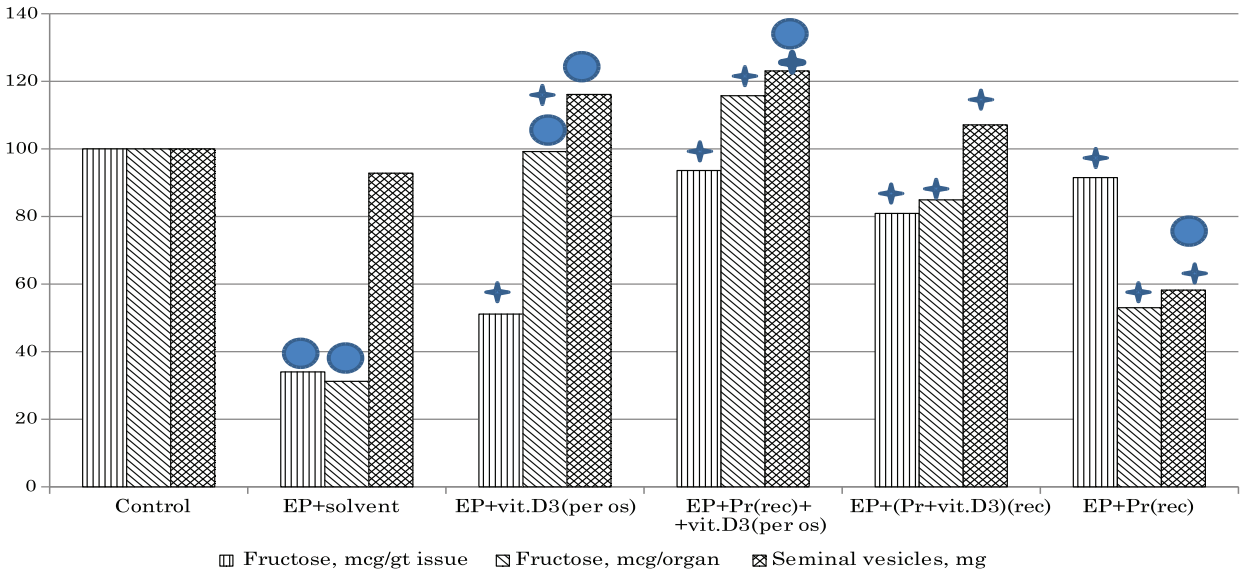

Fig. 1. Fructose concentration and seminal vesicles' masses in males with experimental prostatitis in % to control ($\bar{X} \pm S_{\bar{X}}$).
Notes: ┼) — difference from Control group (false operated);
♦) — difference from EP+solvent group; $p < 0,05$.

portant, in EP + D_3(per os) group of animals the increasing of SV masses by 16 % comparing with Control rats and by 25 % comparing with EP + solvent group has been determined (see Fig. 1; $p < 0,05$). The localization in cell's nucleus of SV receptors for vitamin D_3 explains its ability to influence cells cycles and tissues proliferation in this accessorial gland, which can lead to increasing of organ mass, and due to growing of cells' number — to increasing of fructose concentration. Nowadays, it is known that vitamin D_3 can regulate apoptosis as well as proliferation in testicles [22].

The using of combined composition (Prostatilen with vitamin D_3, per os or rectally) for prostatitis treatment has positively influenced the concentration of fructose in SV. In groups EP + Pr(rec) + D_3(per os) and EP + (Pr + D_3)(rec) fructose concentration has reached Control animals parameters. This index was 2,8 and 2,4 times more than in EP + solvent and Control groups accordingly (see Fig. 1; $p < 0,05$). The calculation of fructose concentration in whole organ in groups EP + Pr(rec) + D_3(per os) and EP + (Pr + D_3)(rec) has shown normalization of parameters, which have reached Control group level (see Fig. 1). Fructose concentration in EP + Pr(rec) + D_3(per os) group was 3,7 times and in EP + (Pr + D_3)(rec) group in 2,7 times higher than in animals which haven't been treated (see Fig. 1; $p < 0,05$). Both groups have shown the increasing of SV mass comparing with EP+solvent: in EP + Pr(rec) + D_3(per os) group this index was 33 % more than in EP + solvent and in EP + (Pr + D_3)(rec) — by 15 % more than in EP + solvent group 15 % (see Fig. 1; $p < 0,05$). In males of EP + Pr(rec) + D_3(per os) group the growing of SV masses by 23 % relative to control animals has been detected (see Fig. 1; $p < 0,05$). It can be explained by androgen influence on the fructose synthesis — Pr supports Ts production in testicles, which in turn, leads to activation of fructose synthesis in this organ. In addition, vitamin D_3 has proliferative activity which stimulates SV active cells growing and causes increasing of gland mass and fructose concentration.

Thereby, the inclusion of vitamin D into complex schemes of prostatitis treatment can be considered to be reasonable because it increases sex hormones level in blood serum and fructose concentration in SV. It can facilitate spermatogenesis and fertility in men with prostatitis.

CONCLUSIONS

1. The modelling of experimental prostate pathology be cryo traumatizing of prostate gland leads to hypotestosteronemia and declining of Testosterone/Estradiol ratio in blood serum of rats' males. The fructose concentration decreases nearly three times in seminal vesicles in animals with experimental prostatitis.
2. The using of Prostatilen as a monotherapy or in combination with vitamin D_3 for experimental prostatitis treatment independently of ways of introduction has led to testosterone level normalization (increasing of testosterone concentration in blood serum,) and fructose content in testicles, but hasn't influenced estradiol concentration.
3. The independent use of vitamin D per os under the condition of experimental prostatitis hasn't led to testosterone secretion recovering, but has increased its influence on the seminal vesicles. The growing of seminal vesicle masses and fructose concentration in them indicates this.
4. The introduction of vitamin D with Prostatilen in complex schemes of treatment is reasonable because has demonstrated positive effect on androgen saturation of organism, has increased fructose concentration in seminal vesicle, which can facilitate spermatogenesis and fecundity.

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THE INFLUENCE OF VITAMIN D_3 USED INDEPENDENTLY, IN COMBINATION OR IN THE PHARMACEUTICAL COMPOSITION WITH PROSTATE CORRECTOR PROSTATILEN ON THE SEX HORMONES LEVELS IN RATS WITH EXPERIMENTAL PROSTATITIS

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Vitamin D is necessary for adequate steroid hormones production and full-fledged spermatogenesis. Its deficiency causes the development of androgen insufficiency and pathospermia, which can lead to subfertility. It may be the background of vitamin D using for treatment of men's reproductive system pathologies. However, the experimental grounding of vitamin D administration on the pathology models that simulate the most widespread variants of infertility is needed to confirm this. Subfertility in men with prostatitis is one of the main causes of infertility.

Aim: the studying of vitamin D using (independently or in complex schemes) for correction of sex hormones levels and fructose concentration in rats' males with experimental prostatitis.

Materials and methods. The correction of pathology has been carried out by vitamin D in sex active Vistar rats with experimental prostatitis which has been induced by cryo traumatizing of ventral part of prostate gland. Vitamin D3 has been used as a monotherapy or in combination with prostate protector Prostatilen (in different ways of introduction), *per os* or in a pharmaceutical composition for rectal use. The masses of seminal vesicles, their fructose concentration and sex hormones levels in blood serum have been determined.

Results. Under the condition of experimental cryo-prostatitis the hypotestosteronemia and decreasing of testosterone/estradiol ration have been detected in rat male blood serum, in the seminal vesicles the fructose concentration has declined nearly three times. The using of Prostatilen as a monotherapy or in combination with vitamin D3 for experimental prostatitis treatment independently of ways of introduction has led to androgen status normalization (increasing of testosterone concentration in blood serum), and fructose content in testicles, but hasn't influenced estradiol concentration. The independent use of vitamin D *per os* under the condition of experimental prostatitis hasn't led to testosterone secretion recovering, but has increased its influence on the seminal vesicles. The growing of seminal vesicle masses and fructose concentration in them indicates this.

Conclusions. The introduction of vitamin D with Prostatilen in complex schemes of treatment is reasonable because has demonstrated positive effect on androgen saturation of organism, has increased fructose concentration in seminal vesicles, which can facilitate spermatogenesis and fecundity.

Keywords: vitamin D, experimental prostatitis, sex hormones level, fructose concentration in seminal vesicles.

ВПЛИВ ВІТАМІНУ D_3, ЗАСТОСОВАНОГО САМОСТІЙНО, В КОМБІНАЦІЇ АБО У ВИГЛЯДІ ФАРМКОМПОЗИЦІЇ З ПРОСТАТОКОРЕКТОРОМ НА РІВЕНЬ СТАТЕВИХ ГОРМОНІВ ЩУРІВ ІЗ ЕКСПЕРИМЕНТАЛЬНИМ ПРОСТАТИТОМ

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Вітамін D є необхідним для адекватної продукції стероїдних гормонів та повноцінного сперматогенезу. Його недостатність сприяє розвитку андрогенодефіциту та патоспермії, що може привести до гіпофертильності. Це наводить на думку про можливість використання вітаміну при лікуванні репродуктопатій чоловіків. Однак для цього необхідне експериментальне обґрунтування застосування вітаміну D на моделях, які відображають найбільш розповсюджені варіанти безпліддя. Гіпофертильність чоловіків із простатитом є однією з провідних причин безпліддя.

Мета: вивчення застосування вітаміну D (самостійно або у комплексних схемах) для корекції рівнів статевих гормонів і концентрації фруктози у самців щурів із експериментальним простатитом.

Матеріали та методи. На статевоактивних щурах популяції Вістар із експериментальним простатитом, який викликали шляхом кріотравмування вентральної частини передміхурової залози, проводили корекцію захворювання за допомогою вітаміну D_3 в якості монотерапії або разом із простатопротектором Простатиленом (при застосуванні різними шляхами) перорально або у вигляді ректальної фармкомпозиції. Визначали масу сім'яних пухирців та концентрацію в них фруктози та рівень статевих гормонів у сироватці крові.

Результати. При експериментальному холодовому простатиті спостерігається гіпотестостеронемія та зниження співвідношення тестостерон/естрадіол у сироватці крові самців щурів, в сім΄яних пухирцях майже в три рази падає концентрація фруктози. Застосування для корекції Простатилену самостійно або у комбінації з вітаміном D_3 незалежно від шляхів введення нормалізує андрогенний стан (підвищення вмісту загального тестостерону в сироватці крові) та фруктози в сім'яних пухирцях та не впливає на вміст естрадіолу. Самостійне застосування вітаміну D_3 при моделюванні експериментального простатиту не призводить до відновлення секреції чоловічого статевого гормону, але сприяє посиленню реалізації його дії на сім'яні пухирці. Про це свідчить зростання концентрації маси сім'яних пухирців та концентрації фруктози в них.

Висновки. Введення вітаміну D разом із Простатиленом в комплексних схемах лікування простатиту є доцільним, так як має позитивну дію на андрогенну насиченість організму, покращує параметри концентрації фруктози у сім'яних пухирцях, що може сприяти покращенню сперматогенезу та запліднювальних властивостей сім'яної рідини.

Ключові слова: вітамін D, експериментальний простатит, рівень статевих гормонів, концентрація фруктози в сім'яних пухирцях.