THE INFLUENCE OF THE RARE-EARTH METALS NANOPARTICLES ON THE RAT’S MALES REPRODUCTIVE FUNCTION IN THE DESCENDING STAGE OF ONTOGENESIS

The anti-aging effects of the rare-earth metals nanoparticles have been studied on the 18-21-month-old rat males. It was shown that the nanoparticles of Gadolinium Orthovanadate activated by Europium (GdVO₄: Eu³⁺) in a dose of 0.33 mg/kg during chronic using (70 days) have demonstrated the anti-aging effects on the old male’s reproductive function manifested by the increasing the sex hormone level and activating the spermatogenesis.

**Key worlds:** nanoparticles of Gadolinium Orthovanadate; reproductive function; spermatogenesis

**INTRODUCTION**

The increasing of the duration of human life in the high developed countries is often associated with the birth rate decreasing and with the changes of the population structure. The part of elderly men is increasing most rapidly. The manifestation of most elderly-aged-associating diseases is going after finishing reproductive period which is accompanied by the androgenic deficiency with men [3, 9]. These changes cause the increasing the amount of the old people with multiple pathologies, that, in its turn, causes the problem of prolonging an active life. The importance of the reproductive health can’t be determined only by the care of the population strength. There is natural slow decreasing of the sex hormone Ts (testosterone) level in the declining year’s men. According to the data of the Massachusetts Adult Search (MMAS) the Ts level is decreasing approximately 0.8 % a year after 30-35 years to the end of life. The development of the age-associated hypogonadal state is closely connected with disturbances in the central hypothalamo-hypophysis system and in the testicles. The amount of the Leidig testosterone-synthesizing cells decreases in the testicles; the density of the luteinising hormone receptors and activity of the Ts synthesizing ferments are lowering. The concentration of the sex steroid binding globulin is increasing in blood, the fraction of the biologically active androgen is decreasing and the ratio Ts/E₂ is changing, the estrogen activity is heightening [18].

Due to Ts versatile activity in the men’s organism the decreasing of the androgen blood level determines not only sexual disturbances but different somatic changes too. It is considered that violation of the sex hormones ratio takes an important part in the age-associated diseases development such as DM (diabetes mellitus) and insulin resistance, determines the activity of the high nervous system, emotion sphere and so on [21]. In fact beginning from 50 years old the glucose blood level is gradually increasing – every 10 years by 0.055 mmol/l. The DM accompanies by the lowering of the total and free testosterone levels and oxidative stress is often developing [12]. There is lack of stimulating insulin action on the Leidig cells Ts production and decreasing those cells reaction to the chorionic gonadotropin introduction under the insulin resistance condition. All those facts quicken the involuion of the reproductive function and make worse the quality of elderly life. Under the Ts deficiency the symptoms complex of the adult organism which requires varied therapy is developing. The polypragmasia may lead to the undesirable interaction between medicines, metabolic disturbances and developing of complications.

Nowadays there is lowered Ts concentration with the youngest men 8.0-12.0 nmol/l (subnormal indices). This accelerated involuion of the reproductive function may be considered as civilization disease [6], which leads to the premature age androgen deficiency and hypofertility with difficult and often empirical treatment [2].

Taking into consideration mentioned facts the correction of the sex hormones disbalance for the decelera-
tion of the organism involution is an important part and expected positive effect of the anti-aging therapy. Moreover, the increasing of the middle-aged part of population requires for the prophylaxis and intensive control of the noninfectious diseases and determines the perceptivity of the innovational approaches and searches of the new nontoxic substances with positive effects on the age processes which would be able to stimulate reproductive function and fertility, to normalize age metabolism, to influence insulin resistance and to support full value of the β-cells function thus showing antidiabetic effects. The substances with endocrinological effects on the human organism might be the most perspective for the anti-aging therapy.

The substances of vanadium should be marked among ones. This element takes up an exceptional position among essential elements, because both of its forms – anionic and cationic – take part in the biological processes [13]. The element is a catalyst in the redox reactions. Vanadium is an inhibitor or regulator of the Na+-K+-ATP-ase, ribonuclease and others ferments. It intensifies the tissues oxygen absorption, catalyzes the phospholipids oxidation by liver ferments and influences the blood glucose level. In addition to their hypoglycemic effect, Vanadium compounds have anti-hypertensive anti-cholesterolemic activities, change food taking throw their influence the hypothalamic neuropeptide Y. This metal is an active to the different organism functions. On the base of animals’ researches it was proven that vanadium using under the conditions of iodine deficiency or goitrogens activity has a positive effects on the thyroid functions. Vanadium inhibits the fat acids and cholesterol synthesis [11]. In experiments with the animal it has been shown that vanadium deficiency in the organism leads to the increasing of the abortion frequency, to thyroid disorders and decreasing lactation [17]. But in the big doses this metal is very toxic for the human organism [10], especially for the reproductive system [15]. It is expected that using the gadolinium ortovanadate in the morphologically normal sperms Cx has been calculated after course (70 days) intaking of the GdVO₄: Eu³⁺ nanoparticles activated by Europium (GdVO₄: Eu³⁺) has been obtained at the Institute for scintillation materials NAS of Ukraine. NP GdVO₄ in dose of 0.33 mg/kg have been given with the feeding avoiding stress influence the high toxicity of the soluble vanadium compound [5, 10] prevents it’s using as insulin-like substance, although, its insoluble salts, especially gadolinium ortovanadate, are nontoxic, but have low bioavailability. It is expected that using the gadolinium ortovanadate in the form of nanocrystals would raise the solubility keeping safe its positive biological effects and nontoxicity. Analyzing above-cited data we might allow that vanadium compounds have anti-aging effects especially with decreasing their toxicity in the form of nanoparticles (NP). The studying of the affectivity of the GdVO₄ nanoparticles using for age changes correction in rat males in the descending stage of ontogenesis as a potential medicines for prevention the age-associated conditions and involution of the reproductive function.

**MATERIALS AND METHODS**

The investigation has been carried out on the Wistar rat males from the vivarium of SI “Institute for endocrine pathology named after V. Ya. Danilevskiy NAMS of Ukraine”. The animals have been kept in the standard conditions of natural light sources, recommended diet and water regime ad libitum. The experiment has been carried out according to the national ethic principles which correlated with thesis of European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

The model for natural aging replaying not only Ts level lowering but also expressive age androgen-depending processes has been used. The influence of the GdVO₄: Eu³⁺ nanoparticles on the reproductive function has been studied on the group of rats (21 males) with fixing age (18 months in the experiment beginning, descending stage of ontogenesis). According to [7] rats after 18 months are related to the pre-elderly period (18-23 months). During experiment the general condition, body weight and glucose blood level have been controlled. The animals have been taken away from the experiment throw the fast decapitation without using narcosis on the 71-st day. Fast decapitation avoids allowing changes of the sex hormone concentration in blood owing to anesthetics action. The indices of the reproductive function have been determined after course (70 days) intaking of the GdVO₄: Eu³⁺ nanoparticles (NP GdVO₄ group). The colloidal water solution of the NP GdVO₄ activated by Europium (GdVO₄: Eu³⁺) has been obtained at the Institute for scintillation materials NAS of Ukraine. NP GdVO₄, in dose of 0.33 mg/kg have been given with the feeding avoiding stress influence the high manipulations with sonde. During animals autopsy the visual inspection and determination of the eternal mass of organs have been carried out. The samples of blood preserving at – 18 °C for determining of sex hormone level (T and E₂) samples have been taken on the 30-th day (from tail vein) and on the 71-st day (during decapitation).

The condition of the reproductive system has been estimated using spermogram indices of the epididymal sperms during microscoping. The concentrations of the epididymal sperms, motility and pathological forms percentage have been determined [7]. The concentration of the morphologically normal sperms C₅ has been calculated [8]. In order to study the morphological indices of the histological sections the testicles have been fixed in the 10 % formaldehyde solution of neutral pH, then cut sections have been dehydrated by passing the tissue trough the increasing concentrations of the ethyl alcohol (from 0 to 100 %). After that the tissue has been embedded into the celidine-paraffin, cut using the microtome and colored with haematoxylin and eosin. On the cut sections of the testicles tissue the morphometric estimating of spermatogenesis indices, the relative quantity of the seminiferous tubules with the spermatogenic epithelium and with the spermatooza in the metaphase of the second part.
The 45-th day of the experiment (20-21-month-old). In exceeded body weight than initial ones beginning from natural aging processes [20]. At the same time it must be confirmed that an increasing body weight is due to the using of the standard diet it may be considered to be as positive effect. During the experiment the glucose blood concentration has been determined. There is no difference between groups of animals as to the initial glucose level; the concentration of the glucose in the whole sample was 5.1 ± 0.2 nmol/l. The glucose level in the control group animals has statistically reliable increased up to 6.0 ± 0.2 nmol/l (135.2 % from initials meanings) when the rats were 19 months. On the 50-th day of the experiment the glucose level was exceeded the initial meanings by the 33.5 % (P < 0.05) and on the 70-th day of the experiment the glucose level was exceeded the age level. The differences with the initial level on the 30-th, 50-th and 70-th days of the experiment were 11.5 (P < 0.05), 8.3 (P < 0.05) and 16.6 % (P < 0.05) accordingly. Thus, the inhibition of the reproduction organs.

The obtained results have shown that investigated substances have no toxic influence on both reproductive and immune systems of old rats.

### Table 1

| Mass                  | Control (n = 7) | NP GdVO₄⁺ (n = 6) |
|-----------------------|----------------|-------------------|
| Testicles, mg         | 35.57 ± 174.4  | 4100.0 ± 194.9*   |
| Seminal vesicles, mg  | 828.6 ± 40.6   | 970.0 ± 88.9      |
| Epididymis, mg        | 1278.6 ± 28.6  | 1320.0 ± 93.0     |
| Ventral prostate gland, mg | 628.6 ± 68.9 | 710.0 ± 64.0      |
| Thymus gland, mg      | 235.1 ± 36.1   | 2126 ± 31.0       |
| Adrenal glands, mg    | 39.4 ± 2.3     | 41.0 ± 3.4        |
| Spleen, mg            | 1171.4 ± 108.5 | 1480.0 ± 158.6    |
| Pituitary gland, mg   | 10.0 ± 1.2     | 8.8 ± 1.3         |

Note: * – Differences between control group (0.05 < P < 0.1).

### Table 2

| Indices                              | Control (solvent) (n = 7) | NP GdVO₄⁺ (n = 5) |
|--------------------------------------|---------------------------|-------------------|
| Concentration, mil./ml               | 42.9 ± 4.4                | 56.2 ± 5.2*       |
| Motility, %                          | 73.1 ± 4.5                | 78.8 ± 4.9        |
| Pathological form, %                 | 14.4 ± 1.4                | 16.0 ± 2.7        |
| Non-motile, %                        | 8.1 ± 1.2                 | 10.6 ± 1.0        |
| C₁₆, mil./ml                         | 33.3 ± 3.8                | 41.2 ± 3.8        |
| Duration of motility, min            | 218.7 ± 14.2              | 258.4 ± 20.9      |

Note: * – Statistical differences between Control group (P < 0.05);
** – The concentration of the morphologically normal and motile spermatozoons.

The obtained results have shown that investigated substances have no toxic influence on both reproductive and immune systems of old rats.

The obtained results have shown that investigated substances have no toxic influence on both reproductive and immune systems of old rats.
According to the Tab. 2, the increasing of the spermatogenesis in the epididymal sperms suspension has been marked under the NP influence.

According to the prior investigations the spermatogenesis with 10-month-old rat males is 35.3 ± 5.6 mil/ml, the part of motile sperms is 69.0 ± 5.4 %, abnormal forms = 17.5 ± 4.7 % and CN = 29.0 ± 5.0 mil/ml. Comparing these indices with the data in Tab. 2 it may be seen that spermogram’s indices of control rat males group (20-20.5-month-old) are the similar.

Comparing these indices with the data in Tab. 2 it may be seen that spermogram’s indices of control rat males group (20-20.5-month-old) are the similar.

The morphometrical characteristics of the spermogram of the rats which took the NP GdVO4 confirmed the visual signs of the spermatogenic epithelium improvements (Tab. 3). The presented data show the evident increasing of the spermatogenic stem cells, the spermatogenesis meioses activity and the index of spermatogenesis with the experimental males. The combination of these signs may be considered to be real stimulation of the spermatogenesis.

The main man’s sex hormone is known to be Ts; it determines sex belonging, regulates the sex development and intensity and character of the sexual behavior with the adults; it regulates the spermatogenesis and metabolism. The level of its derivate E2 synthesized in the target tissue cells by aromatization of the Ts is of great importance because the ratio between these steroids demonstrates the androgen-estrogen correlation and relative androgenization or estrogenization of the organism. Ts synthesizes in the gonads by Leydig cells and gets the blood straight away. Its synthesis is regulated by gonadotropic hormones and is characterized by specific daily rhythm. The blood samples were obtained at one and the same time in 20-th and 60-th day of the nanoparticle feeding. The measuring concentration of steroid showed no significant differences between experimental and control groups on the 20-th day of the experiment (Tab. 4).

The 72 % increasing of the Ts concentration was determined with the males in 60 days of the NP GdVO4 feeding. This index was statistically higher than with the same males or with the control animals on the 20-th day of the experiment. The concentration of E2 in all groups of the experimental animals has no significant differences. Such changes caused increasing the Ts/E2 ratio, they weren’t statistically reliable though. It means, the long lasting application of the NP GdVO4: Eu3+ stimulate Ts synthesis which is demonstrated by the spermatogenesis activating.

Thus, under the condition of the alimental chronic application in dose of 0.33 mg/kg stimulate the spermatogenesis. It has been proved by Ts level increasing, activating the testicles spermatogenic epithelium and by increasing the epididymal spermatoozoons concentration.

The estimating of the obtained results confirms the anti-aging biological activity of the NP GdVO4. Due to the anti-aging effect the NP GdVO4 have hindered the age-dependent hyperglycemia development, inhibited the age-depending increasing of body weight, have positive influence on the spermatogenesis intensity and Ts synthesis with the old rat males.

Indeed, it is very difficult to discuss the obtained results of the NP GdVO4: Eu3+ investigations owing to the unique properties of the nanoparticles and used term
of the researching (70 days). The comparison between the well-known biological properties of vanadium and its salts might be incorrect. First of all, the researchers mark the high toxicity of the vanadium and its salts [5, 10]. In our case from 7 animals of the NP GdVO₄ experimental group only one male died on 67-th day without signs of the toxic injury. During autopsy the increasing of the pancreatic β-cells function is impossible. The answer to this question requires the subsequent specific investigations using the appropriate models.

The significant increasing of the spermatogenesis production accompanied by the testicles mass growing under the influence of the NP GdVO₄ has been determined. It allows to suppose that NP GdVO₄ stimulating effect on the glucose metabolism during NP GdVO₄ application to younger animals that will allow to study NP chronic and gonads toxicity.

The vanadium has demonstrated a positive influence on the glucose metabolism during NP GdVO₄ application [19]. However, to make any conclusions as to any available kind of actions – insulin like or of the prolonging of the pancreas β-cells function is impossible. The answer to this question requires the subsequent specific investigations using the appropriate models.

CONCLUSIONS

1. The nanoparticles of the GdVO₄ in dose of 0.33 mg/kg has demonstrated the significant and long-lasting effects as for glucose metabolism. The inhibition of the age-depending growing body weigh (up to 45-th day) and absence of the increasing glucose blood level (up to 50-th day) have been detected.

2. The positive effect of the nanoparticles of the gadolinium orthovanadate on the reproductive function of old animals by the increasing of the Ts concentration and spermatogenesis activating has been detected.

REFERENCES

1. Безруков В. П. Влияние старения населения на формирование потребностей в финансировании здравоохранения // Матер. консульт. междунар. семинара. – М.: МЗМП РФ, 1995. – С. 149.

2. Бондаренко В. А. Функции печени и андрогенный статус у мужчин с идиопатическими патоспермиями // В. А. Бондаренко, А. С. Минукhin, Е. И. Сверников // Пробл. эндокринол. патол. – 2012 – № 2. – С. 15-19.

3. Верткин А. Л. Возрастной андрогенный дефицит и эректильная дисфункция / А. Л. Верткин, Д. Ю. Пушкин. – М.: Изд. группа «ГЭОТАР-Медиа», 2009 – 176 с.

4. Гланц С. А. Медико-биологическая статистика. – М.: Практика, 1998. – 459 с.

5. Голубев М. А. Сравнительная характеристика ванадий-содержащих соединений, обладающих инсулиноподобным действием / М. А. Голубев, В. К. Городецкий, А. П. Айсинькина // Вопр. мед. химии. – 2000. – № 2. – С. 78-83.

6. Дедов И. И. Возрастной андрогенный дефицит у мужчин [Текст] / И. И. Дедов, С. Ю. Калинченко. – М.: Практ. медицина, 2006. – 240 с.

7. Докладчич доклад докл. засобів [метод. ред. рекоменд.] // За ред. О. В. Стефanova. – К.: Авіценна, 2001. – 528 с.

8. Карпенко Н. О. Интегральная оценка репродуктивной активности самца животных // [Н. О. Карпенко, В. В. Талько, С. Т. Омельчук та ін.] // Укр. біофармац. журн. – 2011. – Т. 13, № 2. – С. 64-68.

9. Куликовский В. Ф. Частичный андрогенодефицит, остеопения и ранние нарушения мицации у стареющих мужчин, связь явлений / В. Ф. Куликовский, Н. В. Олейники // Совр. наукоемкие технол. – 2009. – № 6. – С. 48-49.

10. Пат. 2341528 RU, МПК С 07 F 9/00. Оксованадийный комплекс с гликозидом, проявляющий гипогликемическую активность [Електронний ресурс] / Е. Н. Вергейчик, О. В. Лапочкин, Л. Б. Губанова (RU); заявник та патентовласник ГОУ ВПО Пятигорская государственная фармацевтическая академия Федерального агентства по здравоохранению и социальному развитию (RU). – № 2009116481/04. – Заявл.: 02.05.07. Опубл.: 20.12.08. – Режим доступа: www.fips.ru

11. Скальный А. В. Химические элементы в физиологии и экологии человека [Електронний ресурс] // Режим доступа: http://www.m-kat.ru/ebook.php?file=skalnyy_djvu&page=26

12. American Association of Clincal Endocrinologists. Medical Guidelines for Clinical Practice for the Management of Diabetes Mellitus: AACE Diabetes Mellitus Clinical Practice Guidelines Task Force // Endocrine Practice. – 2007. – Vol. 13, Suppl. 1. – P. 3-66.
13. Baran E. Oxovanadium (IV) and oxovanadium (V) complexes relevant to biological systems // J. Inorg. Biochem. – Vol. 80, Issues 1-2. – P. 1-10.
14. Cam M. C. Mechanisms of vanadium action: insulin-mimetic or insulin-enhancing agent? / M. C. Cam, R. W. Brownsey, J. H. McNeill // Canad. J. Physiol. Pharmacol. – 2000. – Vol. 78, № 10. – P. 829-847.
15. Chandra A. K. Effects of vanadate on male rat reproductive tract histology, oxidative stress markers and androgenic enzyme activities / A. K. Chandra, R. Ghosh, A. Chatterjee, M. Sarkar // J. Inorg. Biochem. – 2007. – Vol. 101, № 6. – P. 944-956.
16. Chandra A. K. Protection against vanadium-induced testicular toxicity by testosterone propionate in rats [Text] / A. K. Chandra, R. Ghosh, A. Chatterjee, M. Sarkar // Toxicol. Mech. Methods. – 2010. – Vol. 20, № 6. – P. 306-315.
17. Domingo J. L. Vanadium: a review of the reproductive and developmental toxicity // Reprod. Toxicol. – 1996. – Vol. 10, № 3. – P. 175-182.
18. Holland J. Testosterone levels and cognition in elderly men: [a review] // Maturitas. – 2011. – Vol. 69, № 4. – P. 322-337.
19. Marzban L. Insulin-Like Actions of Vanadium: Potential as a Therapeutic Agent / L. Marzban, J. H. McNeill // J. Trace Elements in Experim. Med. – 2003. – Vol. 16. – P. 253-267.
20. Thomas M. A. Effects of aging on food intake and body composition in rats / M. A. Thomas, H. B. Rice, D. Weinstock, R. L. Corwin // Physiol. Behav. – 2002. – Vol. 76, № 4-5. – P. 487-500.
21. Yeap B. B. Hormones and health outcomes in aging men // Exp. Gerontol. – 2013. – Vol. 11, № 2. – P. 251-270.

УДК 612.616+591.463.12:612.66+577.1
Н. О. Карпенко, Е. М. Коренева, Е. Е. Чистикова, Н. П. Смоленко, І. О. Бєлкіна, Н. Ю. Селюкова, С. П. Кустова, М. О. Бойко, Ю. Б. Ларьяновська, В. К. Клочков, Н. С. Кавок
ВПЛИВ НАНОЧАСТИНОК РІДКОЗЕМЕЛЬНИХ ЕЛЕМЕНТІВ НА РЕПРОДУКТИВНУ ФУНКЦІЮ
САМЦІВ ЩУРІВ НА НИЗХІДНІЙ СТАДІЇ ОНТОГЕНЕЗУ
На самцях щурів віком 18-21 міс. досліджено антивікові ефекти наночастинок рідкоземельних металів. Показано, що наночастинки гадолінію ортованадату активованого європієм (GdVO₄: Eu³⁺) у дозі 0,33 мг/кг маси тіла при хронічному застосуванні (70 діб) чинять антивікову дію щодо репродуктивної функції старих тварин, у яких зросла концентрація чоловічого статевого гормону та активувався сперматогенез.
Ключові слова: наночастинки гадолінію ортованадату; репродуктивна функція; сперматогенез

УДК 612.616+591.463.12:612.66+577.1
Н. А. Карпенко, Е. М. Коренева, Е. Е. Чистикова, Н. П. Смоленко, І. О. Бєлкіна, Н. Ю. Селюкова, С. П. Кустова, М. О. Бойко, Ю. Б. Ларьяновська, В. К. Клочков, Н. С. Кавок
ВЛИЯНИЕ НАНОЧАСТИЦ РЕДКОЗЕМЕЛЬНЫХ ЭЛЕМЕНТОВ НА РЕПРОДУКТИВНУЮ ФУНКЦИЮ
САМЦОВ КРЫС НА НИСХОДЯЩЕЙ СТАДИИ ОНТОГЕНИЗА
На самцах крыс возрастом 18-21 мес. исследованы антивозрастные эффекты наночастиц редкоземельных металлов. Показано, что наночастицы гадолиния ортованадата активированного европием (GdVO₄: Eu³⁺) в дозе 0,33 мг/кг массы тела при хроническом применении (70 суток) замедляли возрастной рост массы тела и развитие гипергликемии (до 50-х суток) в сравнении с исходным уровнем. Установлено антивозрастное действие вещества относительно репродуктивной функции старых животных, у которых возросла концентрация мужского полового гормона, активировался сперматогенез.
Ключевые слова: наночастицы гадолиния ортованадата; репродуктивная функция; сперматогенез

Адреса для листування: 61002, м. Харків, вул. Алчевських, 10. Тел. 0505826127. E-mail: reproduk@ukr.net.
ДУ «Інститут проблем ендокринної патології ім. В. Я. Данилевського НАМН України»