PROCEEDINGS OF THE INTERNATIONAL CONFERENCE
NANOMATERIALS: APPLICATIONS AND PROPERTIES
Vol. **2** No 4, 04NABM28(4pp) (2013)

The Effects of Chronic Intake of Cerium Dioxide or Gadolinium Ortovanadate Nanoparticles in Aging Male Rats

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(Received 19 June 2013; published online 01 September 2013)

The acquisition of new nanomaterials with unique properties for biomedical application is an essential requirement now days. It is important and necessary to evaluate the safety and the delayed consequences of nanoparticles entry for living organisms. The aim of the present work was to investigate the effects of chronic intake of cerium dioxide and gadolinium ortovanadate nanoparticles of some character in aging male rats. In blind experiment 18 – 20 months age Wistar male rats were fed the colloidal solution (0.5 ml) of the nanoparticles either cerium dioxide or gadolinium ortovanadate ($GdVO_4$:Eu^{3+}) during 70 days. Evolution of emotional state, body mass, glucose homeostasis, level of sex hormone (testosterone and estradiol) has been investigated over a period of experiment. At termination, it was analyzed of spermogram and antioxidant properties of sperm and some tissues. The acute toxicity of nanomaterials were evaluate in outbred mice. No effects of cerium dioxide nanoparticles has been found. $GdVO_4$:Eu^{3+} male rats demonstrated the decrease in accretion of body weight and glucose level, and the less dejection compared with the untreated aging rats. These rats has increased testosterone level, sperm concentration and reduced chemiluminescence of gametes. The acute toxicity test defined GdVO4 nanoparticles as a nontoxic material – LD_{50} > 5 g/kg b·m. Positive influence of $GdVO_4$:Eu^{3+} nanoparticles on metabolic processes, emotional capability and reproductive function of senescent organism could be a basis for formation a new direction of antiaging therapy.

Keywords: Nanoparticles, Gadolinium ortovanadate, Chronic intake, Aging, Rats, Testosterone, Sperm, Glucose, Emotion

PACS numbers: 87.85.Qr, 87.85.Rs

1. INTRODUCTION

The reproductive health is more than just a preservation of population. It is known that the duration of life becomes longer and the age structure of society changes in countries with a limited reproduction of population. The number of elderly people in 2000 at the age over 65 were 3 times higher than in 1950. According to the prediction of experts, this number will grow fourfold by 2050 and total about 1.5 billion people, i.e. approximately 14.5% of the Earth's population. The society will also face the problem of a growing number of elderly people with multiple pathology and therefore, the prolongation of their active life will be urgent. The number of elderly men will increase dramatically and will be 7 times higher than in 1900 and the age group over 85 will be 31 times higher. It is precisely the end of the reproductive period that precedes the aging period.

The main male sex hormone is testosterone that is responsible for the formation of sexual characters, regulation of spermatogenesis and sexual behavior. The level of testosterone starts going down about 0.8% a year after the age of 30 – 35.

Age-related hypogonadism usually leds to disorders of the central regulation links as well as local age-related changes in the testis. Androgenic deficiency gives rise to the set of symptoms such as somatic, endocrine, urino-genital and emotional state disorders that are typical for the old organism. The redistribution of adipose tissue, decrease of muscle tissue could result in the development of insulin resistance in old people. The level of glucose in blood starts gradually growing from the age of 50 by 0.055 mMol/l in 10 years. In some cases, these changes precede the development of type 2 diabetes, that aggravates the associated pathology. According to WHO's prognoses, there will be 300 million people affected by type 2 diabetes (90%) by 2025, which means that diabetes will remain the most widespread disease among the aged people. Diabetes typically causes the decrease of androgenic saturation, the disbalance of pro- and antioxidant processes and the development of oxidative stress that impairs vital functions, speeds up age involution and worsens the quality of life in the middle and elderly age. The treatment of elderly people will face extra problems connected with the aging of nervous system, which decreases the ability to adapt to environmental conditions.

Thus, the elderly people predominantly suffer from polyorganic pathology that requires a multiple medical corrections. However, this polypragmasy is dangerous with undesirable medicine interaction, induction of metabolism disturbance and development of complications.

There is another hazard – a development of premature age androgenic deficiency that increases the likelihood of male infertility. Married couples in modern society tend to postpone having children on many reasons without realizing that they may not have any children at all owing to the age involution acceleration

of reproductive male function – a rather weighty factor of hypofertility.

Such a situation demands a new broad-spectrum antiage medication and new approaches of hypofertility therapy.

It is known that vanadium is an essential element that plays a crucial role in the processes of reproduction, however in big doses the vanadium is harmful for male genital system. In view of the fact that the properties of a substance in a nano scale considerably differ from the properties of the same substance in an ionic scale, an attempt was made to investigate the effect of gadolinium ortovanadate nanoparticles on the reproductive function.

In addition, nanoparticle of cerium dioxide demonstrates an antioxidant properties, which will be useful for keeps the pro/antioxidant balance of aging organism.

The decrease of testosterone level as well as natural changes of other androgen dependent processes accompanies the age involution of organism. Therefore, the model of natural aging was chosen in order to observe the effects a long treatment of gadolinium ortovanadate or cerium dioxide nanoparticles on the responses of the some androgen dependent processes. We expect, this study give possibility to develop some ideas of a possible physiological mechanism of their action.

The aim of this research is to investigate some metabolic parameters, emotional state and reproductive functions of the rats on a descending stage of ontogenesis (18 – 20 months old) with chronic treatment of cerium or vanadium nanoparticles.

2. MATERIAL AND METHODS

Our experiments were carried out in summer on Wistar male rats that were initially 18 months old following the National Ethical Principles of Experiments on Animals. 21 male rats were randomized into 3 groups that were called Control, $GdVO_4$ and CeO_2. Each rat was kept in its own cage with food and water ration *ad libitum* at air temperature 21 – 22°C. Encoded by developers colloidal water solutions of nanoparticles ($GdVO_4$ and CeO_2) as well as solvents were investigated in a "blind" experiment. 300 – 350 mg of dried bread moistened in 0.5 ml of the test solution was given to the rats 5 – 6 times a week on empty stomach in the morning for 70 days. The dose of nanoparticles was 0.1 mg/animal a day or 0.33 mg/kg of body mass. The control group was given water.

Monitoring the general conditions of the rats, we studied their body weight changing, emotional state (Tail suspension test), level of fasting glycemia, sexual reactions (tests of sexual behavior) and collected blood for hormonal analysis. The rats were killed on day 71 by quick decapitation. Then we determined the characteristics of the suspension of epididimal sperms, organs of immune (thymus, spleen) and reproductive (testis, seminal vesicle, the ventral part of prostate gland, epididymides, and hypophysis) systems as well as adrenal glands were removed from each animal and weighed. The sex hormone levels (testosterone and estradiol) and the histology of testis were determined. All manipulations were performed by operators who were blind to the treatment group.

Along with physiological indices, we studied peroxide processes in liver, testis and sperm suspension by the registration of spontaneous and initiated chemiluminescence (CL) values. The chemiluminescence was induced by Fenton's solution. The emission was registered by a device Lum-5773.

We determined the acute toxicity of leader compound on outbred mice weighing 20 – 22 g by administrating per os a maximum possible volume of the solution with a maximum possible concentration of the substance (15 g/l), i.e. 375 mg/kg b. m. in a dose, which is 1100 times greater than in a chronic experiment.

Comparison of the results with the initial parameters of the sampling and the data of control group was made using t Student's and F Fisher's tests. We also used Chi square for categorical variables. P values < 0.05 were considered statistically significant.

3. RESULTS

One can see that the rats of all groups after 2 months of the experiment, i.e. being 20 – 21 months old, increased statistically significant their body weight by 7.1 – 8.7 % in comparison with 18 months old rats. On the one hand, we can say that there is no general toxicity effect at chronic treatment of the test substances, but on the other, considering the standard ration for feeding the rats, we can assume that the increase of body weight was due to a natural aging process. The rats of control group demonstrated this increase from the 45th day of the experiment with glucose concentration going up in peripheral blood (from the 30th day, from 5.1 ± 0.2 up to 6.0 ± 0.2 mMol/l, by 135.2 %, $P < 0.05$). Then the level of glycemia exceeds the initial index on the 50th day by 33.5 % and on the 70th day of the experiment by 18.5 %.

The increase of body weight of the $GdVO_4$ group reached a statistical significance only after 60 days with the level of glycemia remaining on the initial level on the 30th and 50th day of the experiment. Moreover, on the 30th day this value was statistically reliably lower than in the control group and only on the 70th day reached the level of the adult control group. It shows that the development of age-related hyperglycemia slows down, which means that there is a certain relationship between the stability in normal body weight and normoglycemia in this group.

The increase of body weight of the rats of CeO_2 group occurred earlier from the 30th day with their glucose concentration exceeding the initial indices (just like for the rats of control group) on the 30, 50 and 70th days by 42.9, 23.5 and 15.8 %, respectively.

Considering the unfavorable effect of overweight and hyperglycemia on the quality of life and development of various illnesses, such an act of CeO_2 is undesirable but the slowdown in the increase of body weight and hyperglycemia under chronic treatment by $GdVO_4$ is positive.

The length of immobility period in a behavioral test allows us to judge an emotional state of the rats. At the very beginning of the experiment, it was 8.6 ± 1.4 s. The length of immobility decreased for all rats in the second test on the 20th day, which shows that they got

accustomed and gained some experience. This decrease up to 58.1 and 45 % was statistically significant for groups Control and GdVO4, respectively. As for group CeO2, its 19 % decrease was unreliable.

The immobility time of the rats did not change on the 60th day, which probably means that this length is minimal for the male rats of this age. Group $GdVO_4$ had even smaller index up to 44.2 % of the initial one and became less statically significant than that of the control rats. The increase of immobility time in the test being associated with a development of depression syndrome, the decrease of immobility period may indicate the decrease in apathy of the rats, i.e. a positive dynamics of their emotional state, which in case of humans may reveal as an enhancement of will to live. The decrease of the index in group CeO_2 was unreliable (77 % of the initial index).

Sexual behavior of the male rats considerably changes with age. A slight modification in the natural decrease of the sexual activity in groups $GdVO_4$ and CeO_2 was observed in our experiment. Some number of the rats remained capable for copulation on the level of sexual activity typical for the rats of a younger age.

The reaction of generative and incretory functions of testis on the nanoparticle chronic treatment was estimated by the weight of reproductive system organs, the state of spermatogenesis and the level of sexual hormones.

The weighing of thymus, spleen and adrenal gland did not reveal a statistically significant difference between the groups. However, the weight of the rats' testis in group $GdVO_4$ was slightly increased in comparison with control group ($0.05 < P < 0.1$), which was in concordance with the increase of spermatozoa concentration, for it is precisely the cells of spermatogenous epithelium and the content of seminiferous tubules that determine the weight of the organ.

It is worth noting that the increase of spermatozoa concentration during the 70-day experiment is due to the activation of spermatogenous epithelium cell division on the initial stage of nanoparticle treatment. Further research is needed to learn about the delayed action of the nanomaterial. However, the increase in testis weight lets us believe that the activating effect of $GdVO_4$ nanoparticles is rather long and not immediately apparent. This is perhaps the reason why papers devoted to the influence of vanadate on organism have not mentioned its effect on the reproductive functions.

The rest of indices of spermatozoa suspension did not differ from the control group. Perhaps other models of reproductive function disorders can give us more information about the nanopartilce action target.

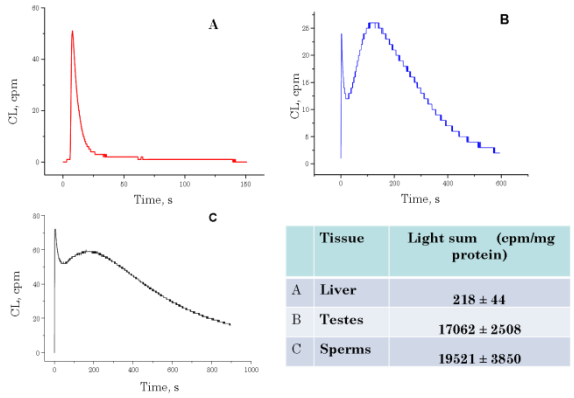
There was no positive effect on the spermatogenesis in group CeO_2, on the contrary, there was a tendency to the increase of anomalous spermatozoa forms, though it may not affect the fertility of the rats.

The level of sexual hormones on the 30th day did not differ in the groups. However, there was an increase in the rats' testosterone concentration of group $GdVO_4$ on the 70th day, which led to androgen-oestrogen ratio increase, i.e. to a relative hyperandrogenisation. It can be assumed that the positive effect on the reproductive function state in this group was due to the influence of $GdVO_4$ nanoparticles on the level of male sexual hormone. Perhaps it can account for the observed antidepressant action. Further research can give us a more precise assessment of the point of substance application directly to hormone-producing testicular cell or the central regulating mechanisms.

Table – Changes of testosterone level (nMol/l) in different periods of experiment

Groups	Days of experiment		P
	20th day	70th day	
Control	13.0 ± 2.8	12,8 ± 1.8	
$GdVO_4$	11.6 ± 2.5	20,0 ± 4.1	$P < 0.05$
CeO_2	14,5 ± 2.8	16,7 ± 2.6	

Fenton's reaction is often used for evaluation of the antioxidant properties of samples. We found that the studied tissues differ in the dynamics of induced chemiluminescence process, which means that they differ in the supply of endogenous antioxidants and the activity of enzyme system of antioxidant protection (Fig. 1).



	Tissue	Light sum (cpm/mg protein)
A	Liver	218 ± 44
B	Testes	17062 ± 2508
C	Sperms	19521 ± 3850

Fig. 1 – Chemiluminescent reaction kinetics for the different samples of tissue

Liver tissue turned out to be the most stable to lipoperoxidation, as the second slow flash was not present in the graph. Testis and sex cells are the most sensitive to the induction of lipid free-radical oxidation, which can be explained by a high concentration of polyunsaturated fat acids. The kinetics curves show that the initiated processes of free-radical oxidation have the longest duration in spermatozoa.

The light output of the initiated luminescence is an integrated index that characterizes the total process intensity and is one of the most informative criterions in evaluation of oxidation disbalance at pathological states.

It should be noted that the investigated nanoparticles did not affect the CL light output in liver and testis (Fig. 3). However, for the spermatozoa of the male rats treated with nonoparticles of GdVO4 this index statistically reliably decreased, which indicates a decrease in oxygen radical generation under administration of Fenton's reagent – a clear evidence of a big antioxidant potential of the rats' sex cells in this group.

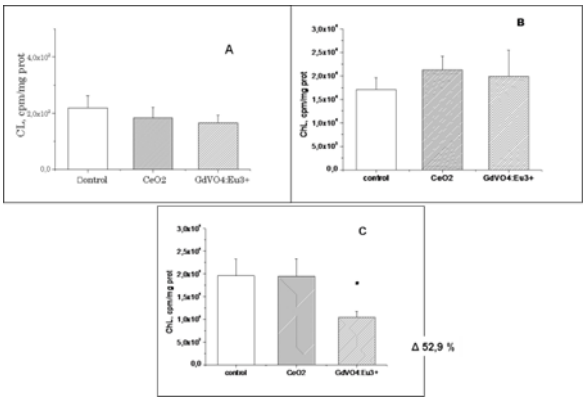


Fig. 2 – Luminol-dependent chemiluminescence in the different samples of tissue

We should like to emphasize the importance of such changes, since according to the data of many studies there is a negative correlation between the intensity of oxygen radical formation and functional spermatozoa characteristics. Besides, the CL intensity of sperms is has been recently used for unclear genesis infertility diagnostic tests.

The revealed ability of $GdVO_4$ nanoparticles to increase an androgenic saturation of organism with spermatogenesis activation and the decrease of ChL intensity of sex cells give evidence of a positive influence of the compounds on the reproductive function. However, further research is needed in order to explain how nanoparticles affect nervous system, metabolic processes and redox balance in various tissues.

4. CONCLUSION

1. The chronic application of CeO_2 nanoparticle did not affect the state of old male rats.

2. Nanoparticles $GdVO_4$ had an apparent and long effect on carbohydrate metabolism, keeping up glycemia on a normal level (up to 50 days) and slowing down the age-related increase of body weight (up to 60 days).

3. The chronic application of nanoparticles $GdVO_4$:Eu^{3+} had a positive effect on their emotional state and sexual reactions of the old male rats. Some acceleration of their behavioral reactions was observed.

4. It was revealed that $GdVO_4$ nanoparticles stimulated the reproductive function of the old male rats, increasing the testosterone concentration, activating spermatogenesis and elevated the antioxidant activity of mature sperms.