EFFECTS OF METFORMIN AND LUTEINIZING HORMONE RECEPTOR AGONISTS ON STEROIDOGENESIS AND SPERMATOGENESIS IN RATS WITH TYPE 2 DIABETES WITH THEIR SEPARATE AND COMBINED ADMINISTRATION

A.A. Bakhtyukov,[1*] K.V. Derkach,[1] A.M. Stepochkina,[1] V.N. Sorokoumov,[1,2] L.V. Bayunova,[1] I.A. Lebedev,[1] A.O. Shpakov[1]

[1]I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia; [2]Saint Petersburg State University, St.-Petersburg, Russia.

E-mail: bahtyukov@gmail.com

ResearchGate: https://www.researchgate.net/profile/Andrey-Bakhtyukov-2

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Abstract

Background: Improving the male reproductive functions in type 2 diabetes mellitus (T2DM) is one of the urgent problems of endocrinology. For this, along with the drugs that enhance testosterone synthesis, such as human chorionic gonadotropin (hCG) and low-molecular-weight allosteric luteinizing hormone receptor (LHR)-agonists, the drugs that normalize glucose homeostasis and insulin sensitivity, such as metformin, can be used. **Objective**: To study the effectiveness of separate and combined administration of metformin, hCG and allosteric LHR-agonist 5-amino-*N*-*tert*-butyl-2-(methylsulfanyl)-4-(3-(nicotinamido)phenyl)thieno[2,3-d]pyrimidine-6-carboxamide (TP03) developed by us on steroidogenesis and spermatogenesis in male T2DM rats. **Methods:** T2DM was induced by high-fat diet (15 weeks) and streptozotocin (25 mg/kg). Metformin treatment (120 mg/kg/day) of diabetic rats was carried out during 4 weeks, while treatment with hCG (20 IU/rat/day) and TP03 (15 mg/kg/day) was performed in the last five days of experiment. **Results:** In diabetic rats, metformin normalized spermatogenesis and partially restored testicular steroidogenesis. hCG and TP03 significantly increased the testosterone production and partially restored the number of epididymal spermatozoa and their progressive movement. When metformin and LHR-agonists used together, on the first day of treatment with LHR-agonists metformin significantly enhanced their steroidogenic effects, but on the 3-5th day, its potentiating effect disappeared. In metformin-treated rats, LHR-agonists made no additional contribution to improving spermatogenesis, which was fully restored by metformin. **Conclusion:** In T2DM rats, metformin therapy and LHR-agonists improve steroidogenesis and spermatogenesis, with metformin being more effective in restoring spermatogenesis. Their combined use leads to a significant increase in the steroidogenic effect of LHR-agonists in acute but not chronic administration.

Results

Table 1. The body weight, the blood fasting levels of glucose, insulin, leptin, glycated hemoglobin (HbA1c) and the insulin resistance index in the control, diabetic and metformin-treated rats

Metabolic Parameters	Control	T2DM	T2DM+MF
Body weight, g	352.2 ± 3.9	402.2 ± 5.2 *	361.3 ± 5.5**
Glucose, mM	4.34 ± 0.08	6.57 ± 0.12 *	5.64 ± 0.11 **
Insulin level, ng/mL	0.61 ± 0.04	0.96 ± 0.05 *	0.66 ± 0.04 **
IR, arb. units	2.68 ± 0.18	6.40 ± 0.42 *	3.81 ± 0.28 **
HbA1c, per cent (%)	4.52 ± 0.08	7.07 ± 0.14*	5.56 ± 0.15 **
Leptin level, ng/mL	1.81 ± 0.09	3.49 ± 0.11*	2.00 ± 0.07**

Note. The duration of MF treatment (120 mg/kg/day) was 4 weeks (before treatment of animals with LHR-agonists). * – the differences with control rats are significant at $p<0.05$; ** – the differences between the T2DM and T2DM+MF groups are significant at $p<0.05$. The data are presented as the $M \pm SEM$, n=15.

Table 2. The effect of metformin treatment and 5 days LHR-agonists administration on sperm parameters of diabetic rats

	Control	Control+TP03	Control+hCG	T2DM	T2DM+TP03	T2DM+hCG	T2DM+MF	T2DM+MF+TP03	T2DM+MF+hCG
Sperm count, 10(6)/ml	22.0 ± 1.5	27.6 ± 2.9	34.0 ± 2.3*	19.0 ± 3.0	25.4 ± 3.5	31.0 ± 5.5	34.2 ± 3.1*#	27.4 ± 2.2	28.8 ± 3.9
Proportion of motile sperm, %	53.6 ± 4.3	59.6 ± 2.6	60.8 ± 3.1	43.6 ± 3.1	56.0 ± 2.0	61.4 ± 3.2*#	58.2 ± 3.7#	53.0 ± 9.9	59.0 ± 4.6#
Proportion of sperm with progressive motility, %	36.6 ± 1.4	45.0 ± 3.4*	48.2 ± 0.9*	22.2 ± 5.1*	35.6 ± 1.5#	46.2 ± 2.7#	40.6 ± 3.1#	38.6 ± 3.1#	41.0 ± 2.4#
Proportion of defective sperm, %	15.4 ± 1.8	17.8 ± 1.9	17.6 ± 2.4	32.0 ± 3.2*	26.4 ± 2.9	25.6 ± 2.4#	21.2 ± 2.3#	20.2 ± 2.1	23.8 ± 2.3

Note. * – the differences from the Control group are significant at $p<0.05$, # – the differences from T2DM group are significant at $p<0.05$. The data are presented as the $M \pm SEM$, n=5.

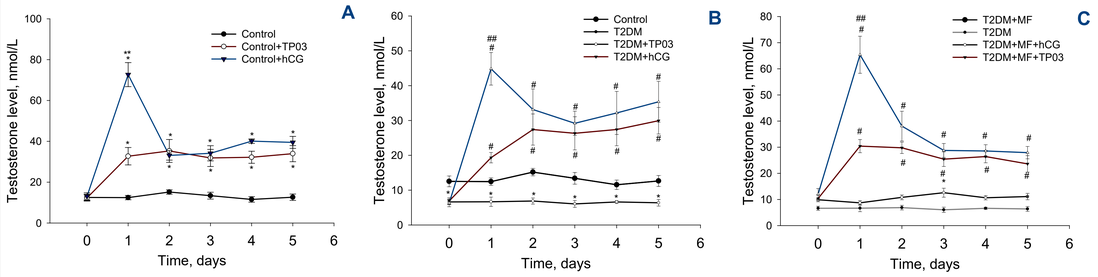

Figure. The effect of LHR-agonists administration on testosterone level in control, diabetic and metformin-treated male rats
The duration of MF treatment (120 mg/kg/day) was 4 weeks. LHR-agonists were administrated during 5 days on last week of the MF-treatment. **A** – effects of TP03 (15 mg/kg, i.p.) or hCG (20 IU/rat, s.c.) on testosterone level in control rats; **B** – effects of TP03 or hCG on testosterone level in T2DM rats; **C** – effects of TP03 or hCG on testosterone level in metformin-treated rats. The blood samples were collected daily 180 min after hCG or TP03 administration.
* - The differences from Control group are statistically significant at $p<0.05$; ** - the differences between groups Control+TP03 and Control+hCG, # - differences from T2DM (**B**) or T2DM+MF (**C**) groups are statistically significant at $p<0.05$, ## - the differences between T2DM+TP03 and T2DM+hCG or T2DM+MF+TP03 or T2DM+MF+hCG are statistically significant at $p<0.05$. $M \pm SEM$, n = 5.

Introduction

Type 2 Diabetes mellitus (T2DM) is a disease that, among the general metabolic disorders, is characterized by dysfunctions of the male reproductive system, such as androgen deficiency and impaired spermatogenesis. The key causes of these disorders are insulin resistance, dysfunction of the hypothalamic-pituitary-gonadal axis, and oxidative stress. Widely used drug metformin shows promising results in improving the steroidogenic function of the testes in animals. However, the data for such treatment regimens still require substantial supplementation and validation. For the treatment of androgen deficiency, preparations of human chorionic gonadotropin (hCG) and recombinant luteinizing hormone (LH) are used. But using of the gonadotropins leads to development of side effects such as a decrease in the sensitivity of Leydig cells to the action of endogenous LH. In recent years, new low molecular weight allosteric LH receptor agonists have been actively developed. The LHR-allosteric agonists interact with their receptor at a different site than gonadotropins and activate steroidogenesis without causing a decrease in LH sensitivity. The one of the most active LH receptor allosteric agonists is the thienopyrimidine derivative TP03, developed and studied by our research group. The aim of this work was to study the effect of LH agonists hCG and TP03 on testosterone levels and sperm activity parameters in male rats with experimental T2DM and during treatment with metformin.

Methods

Male Wistar rats (3-months old) were used in the study. Experimental T2DM was induced with 10 weeks of high fat diet followed by injection of a low dose of streptozotocin (STZ) (25 mg / kg). Control animals housed on normal feed and received citrate buffer instead STZ. Two weeks after STZ, the animals were randomized to the Control, T2DM and T2DM + MF group. Animals in the T2DM + MF group received oral metformin at a dose of 120 mg / kg for 4 weeks. After that, the metabolic parameters and effects of metformin treatment were measured (Table 1), LHR-agonists TP03 (15 mg / kg, i.p.) and hCG (20 ME / rat, s.c.) were administered for 5 days on the last week of MF-treatment. Testosterone level was measured before treatment and daily during experiment 180 min after drugs administration using ELISA. Spermatogenesis parameters were measured on the fifth day of the experiment.

Discussion and conclusion

T2DM male rats had an increase in body weight, fasting blood glucose, insulin, index, glycated hemoglobin, and leptin levels and IR index (Table 1), as well as a decrease in testosterone level and a decrease in sperm quality parameters (Table 2, Figure), while metformin treatment significantly improved all these parameters. Restoration of testosterone level and spermatogenesis parameters with metformin may result from improved insulin sensitivity. Treatment of rats with LH-agonists led to an increase in testosterone level and an improvement in spermatogenesis parameters both in the control and in the untreated and MF-treated diabetic groups. At the same time, the treatment with hCG led to a decrease in the stimulating effect on the testosterone level on the 2-5 days of treatment, which did not occur with the TP03-treated groups. This is due to a decrease in the sensitivity of the Leydig cells of the testes to the long-term action of gonadotropins. At the same time, 4 weeks of treatment with metformin did not significantly increase the effects of LH-agonists on testosterone levels and spermatogenesis parameters. Thus, in rats with T2DM, treatment with metformin and LH-agonists restored androgenic function and improved sperm quality, but metformin did not significantly enhanced the effects of LH-agonists after chronic administration.