THE IMPACT OF SUCCINATE DERIVATIVE PHENSUCCINAL ON MITOCHONDRIAL FUNCTION AND REDOX STATUS IN THE HEART OF RATS WITH TYPE 2 DIABETES[*]

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Diabetes mellitus (DM) is a chronic, non-communicable disease with pandemic prevalence and the seventh leading cause of the death worldwide. Estimations by the World Health Organization reported that a total of 9.3 % of the world population were affected by diabetes in 2019 [1].

The main cause of morbidity and mortality in diabetic patients are cardiovascular complications. DM increases the risk of future heart failure up to fivefold independent of the macrovascular complications of diabetes (including hypertension, coronary artery disease and atherosclerosis). This phenomenon defined as diabetic cardiomyopathy (DCM) includes impairments in cardiac metabolism, structure and function induced by hyperglycaemia and insulin resistance [2].

The mitochondria are the center of metabolism, and emerging data indicates that mitochondrial dysfunction may play a critical role in the pathogenesis of insulin resistance, type 2 DM (T2DM) and diabetes-associated cardiovascular diseases, including DCM [3, 4]. Mitochondrial dysfunction is recognized as a decrease in the ATP production/respiration rate, oxidative stress, and impaired redox- and Ca^{2+}-dependent intracellular signalling. An overproduction of reactive oxygen species (ROS) in the respiratory chain is one of the earliest manifestations of mitochondrial dysfunction, which triggers further metabolic imbalances, leading to a decrease in insulin sensitivity and progression of comorbidities [4]. Therefore, it is suggested that pharmacological action aimed at enhancing the mitochondria oxidative capacity and reducing ROS overproduction in the respiratory chain may be effective for the prevention and correction of complications associated with T2DM.

Succinic acid, a Krebs cycle intermediate that activates complex II of the electron trans-

port chain (ETC), via succinate dehydrogenase-induced generation of FADH2, may be particularly useful in type 2 diabetes and obesity, which have been suggested to have a deficiency in complex II [5].

It was previously reported that the low-toxic succinate derivative phensuccinal (beta-phenylethylamide-2 hydroxy-succinanylic acid, Phe) has an antidiabetic effect, being able to affect simultaneously the main pathogenic dia-betic links (increasing both insulin secretion and action) in different experimental models of insulin deficiency and insulin resistance [6, 7]. In addition, Phe possesses antiatherogenic, antioxidant and anti-inflammatory action [8].

The aim of the present work was to study the effect of Phe on the mitochondrial function and oxidative status in the heart of rats with T2DM.

MATERIALS AND METHODS

All chemicals used were of analytical reagent grade quality and purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phe was provided by Public Joint Stock Company «Borshchahivskiy CPP» (Kyiv, Ukraine).

The present study was approved by the bioethics committee of the «V. Danilevsky Institute for Endocrine Pathology Problems of the National Academy of Medical Sciences of Ukraine» (Kharkiv, Ukraine) and performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

The experiment was performed on 24 male Wistar rats (12-week-old, 130–160 g body weight (b.w.)), which were housed in Plexiglas cages (3 animals per cage) at a temperature of (22 ± 1) °C in a constant 12-hour light/dark cycle. The animal model of T2DM induced by a high calorie diet combined with multiple low-dose streptozotocin (STZ) injections was used. Experimental rats (n = 24) were fed the high calorie diet containing 15 % lard, 25 % sucrose, 1 % bile salts and 59 % standard feed for 10 weeks. Control intact rats (n = 8) were fed a standard diet during 10 weeks. The animals had free access to water. In four weeks, experimental rats were i.p. injected with STZ (25 mg/kg b.w.) once per week for two weeks [9]. Control rats received citrate buffer following the same scheme. Seven days after the last STZ injection, basal glucose was measured in all animals, and experimental rats were di-vided into two groups: untreated diabetic rats (Diabetes, n = 8), diabetic rats treated with Phe (Diabetes + Phe, n = 8) in a dose of 50 mg/kg b.w. once per day intragastrically by gavage for four weeks after diabetes induction. Untreated diabetic rats received vehicle along the same scheme. The animals were sacrificed according to the protocol of the ethics committee.

Tail blood glucose levels were measured using a glucose analyzer Eksan-G (Analita Firm Joint Stock Company Ltd., Vilnius, Republic of Lithuania). Mitochondria were isolated by conventional procedures [10]. Redox status of rats' heart mitochondria was estimated by determination of ROS production, glutathione (GSH) level and activity of antioxidant enzymes: manganese superoxide dismutase (Mn-SOD), glutathione peroxidase (GPX) and glutathione reductase (GR) [11–15]. Mitochondrial function was determined by activity of aconitase, succinate dehydrogenase (SDH) and cytochrome C oxidase (COX) in rats' cardiomyocytes [16–18]. Mitochondrial protein was determined by the Lowry method modified by Miller, with BSA as the standard [19].

Data are presented as mean ± standard error of mean (SEM). The Shapiro-Wilk test was used to test normality of data distribution. For multiple comparisons of data with a normal distribution, a parametric one-way analysis of variance (ANOVA) was performed and the Student-Newman-Keuls method was used to test differences in means. Values were considered statistically significant at $p < 0.05$.

RESULTS AND THEIR DISCUSSION

As shown in Table 1, the basal glucose level in diabetic rats was significantly higher compare to control rats. It was found that the ad-ministration of Phe to diabetic rats significant-ly decrease basal hyperglycaemia compared to the group receiving vehicle.

Table 1

Impact of Phe on basal glucose level in diabetic rats,
($\bar{X} \pm S_{\bar{X}}$), n = 8

Indexes	Control	Diabetes	Diabetes + Phe
Basal glucose level, mmol/l	4.45 ± 0.16	8.21 ± 1.14*	5.51 ± 0.24#

Note:

Data are shown as mean ± standard error of the mean (SEM).

* — p < 0.05 vs «Control»,

— p < 0.05 vs «Diabetes».

Table 2

Impact of Phensuccinal on redox status of isolated heart
mitochondria of diabetic rats, ($\bar{X} \pm S_{\bar{X}}$), n = 8

Indexes	Control	Diabetes	Diabetes + Phe
Production of ROS, H_2O_2/min/ mg protein	0,17 ± 0,02	0,38 ± 0,04*	0,15 ± 0,02#
Mn-SOD activity, a.u./ mg protein	16,97 ± 1,10	29,49 ± 1,94*	16,27 ± 1,70#
GPX activity, µmol GSH/min/ mg protein	11,32 ± 1,12	28,01 ± 3,22*	12,94 ± 1,62#
GSH content, nmol/ mg protein	5,33 ± 0,31	2,89 ± 0,29*	5,37 ± 0,29#
GR activity, µmol NADPH/ min/mg protein	4,42 ± 0,29	6,02 ± 0,28*	5,73 ± 0,60*

Note:

Data are shown as mean ± standard error of the mean (SEM).

* — p < 0.05 vs «Control»,

— p < 0.05 vs «Diabetes».

We can suggest that the hypoglycemic effect of Phe may be due to its stimulating effect on insulin secretion. It is known that impairment of glucose-induced insulin secretion in T2DM may be caused by GLUT 2 underexpression, glucose 6-phosphatase overactivity, the glucokinase gene mutation, FAD-linked glycerophosphate dehydrogenase deficiency and a mitochondrial DNA defect induced by glucotoxicity in the pancreatic beta cells. It was proposed that the methyl esters of succinic acid and related molecules may bypass these defects in glucose transport, phosphorylation and further catabolism and, hence, to stimulate both proinsulin biosynthesis and insulin release in T2DM. Treatment with succinic acid derivative have been shown to manifest insulinotropic properties resulting in lowering of blood glucose and glycosylated hemoglobin in diabetic rats [20].

It is known that mitochondrial dysfunction in T2DM can lead to excessive free-radical production, decreased oxidative phosphorylation efficiency, and reduced mitochondrial ATP production due to interruptions in the ETC [20]. We revealed that the intensity of ROS production during respiration in isolated heart mitochondria of diabetic rats was more than two times higher as compared with the control group (Table 2). The administration of Phe completely normalized this parameter in contrast to animals that received vehicle.

These findings agree with previous studies, which have found that succinic acid monomethyl ester, possesses antioxidant properties, leading to improved redox balance in a murine model of type 1 diabetes [21].

It was found that an increase in ROS production during respiration was accompanied by 74% and 147% increased activities of mitochondria Mn-SOD and GPX, respectively, in the heart of rats with T2DM. It may be result of Keap1/E2-NF redox-sensitive signaling system activation by excessive ROS level, which

Table 3

**Impact of Phensuccinal
on the functional status of isolated heart mitochondria
of diabetic rats, ($\overline{X} \pm S_{\overline{X}}$), n = 8**

Indexes	Control	Diabetes	Diabetes + Phe
Aconitase activity, nmol NADPH/min/mg protein	320,0 ± 20,7	138,5 ± 13,5*	269,6±38,4#
SDH activity, nmol oxidized succinate/ min/mg protein	23,70 ± 2,04	14,65 ± 1,13*	25,05±2,20#
COX activity, μmol oxidized DPD/min/mg protein	11,05 ± 0,65	11,77 ± 1,05	10,87±0,94

Note:

Data are shown as mean ± standard error of the mean (SEM).

* — $p < 0.05$ vs «Control»,

\# — $p < 0.05$ vs «Diabetes».

induces expression of many antioxidant enzymes genes including Mn-SOD and GPX [22]. Thus, negative feedback is carried out, which eliminates redox signaling at the level of ROS and prevents their excessive accumulation. The use of Phe resulted in normalization of these enzymes activity (Table 2).

It was found that the level of GSH in the heart mitochondria of diabetic rats was reduced by more than 40 % compared to the control group, while the activity of GR in rats with T2DM was significantly higher than in animals without diabetes (Table 2). The administration of Phe prevented a decrease of GSH level in cardiac mitochondria of diabetic rats (Table 2).

Nevertheless, Phe did not affect the increase in GR activity induced by the development of T2DM (Table 2).

Taking into account the mechanism of regulation of the activity of Mn-SOD and GPO, it can be assumed that the effect of Phe on mitochondrial function is based on its ability to reduce the production of ROS in the respiratory chain to a greater extent than to stimulate the antioxidant defense system.

It was shown that $O_2^{\cdot-}$ can regulate energy metabolism in mitochondria by modulating the activity of aconitase, an enzyme of the tricarboxylic acid cycle that catalyzes the conversion of citrate into isocitrate [23].

An increase in ROS production in the mitochondrial respiratory chain under conditions of a high concentration of reducing equivalents and a low demand for ATP leads to inactivation of aconitase and, as a result, to the accumulation of citrate in the mitochondrial matrix and induction of insulin resistance and other metabolic derangements.

We revealed that the activity of aconitase in the heart mitochondria was reduced by more than 50% in rats with T2DM compared to the control group (Table 3).

The treatment with Phe led to restoration of aconitase activity in diabetic animals in contrast to diabetic rats receiving vehicle (Table 3).

The study of Phe effect on the SDH activity was of particular interest because it is both a component of the Krebs cycle and the mitochondria respiratory chain (complex II). It was found that activity of SDH, in the heart mitochondria of rats with T2DM was reduced by almost 40 % as compared with the control group. Our results confirmed that the deficiency in complex II of ETC might be one of the causes of cardiac mitochondrial dysfunction in T2DM. The use of Phe prevented a decrease in SDH activity in the cardiac mitochondria of diabetic animals, and the parameter did not differ from the intact rats (Table 3).

It should be noted that neither the development of diabetes nor the administration of Phe affected the activity of another component of the respiratory chain, COX (complex IV) (Table 3).

We can suggest that Phe ameliorate mitochondrial dysfunction decreasing oxidative stress and preventing deficiency in complex II of ETC in the heart of rats with T2DM.

CONCLUSIONS

Our results demonstrated that Phe inhibited oxidative stress in isolated cardiac mitochondria decreasing ROS production, increasing GSH level and normalizing antioxidant enzymes activity in the heart of rats with T2DM. The treatment with Phe improved metabolic activity of the heart mitochondria inducing aconitase activity in cardiomyocytes. In addition, Phe ameliorated cardiac mitochondrial dysfunction preventing deficiency in complex II of ETC in diabetic animals.

The data of the present study confirmed the positive effect of Phe on redox homeostasis and functional state of the heart mitochondria in rats with T2DM. We suggest that the use of Phe may contribute to the amelioration of diabetic cardiovascular complication.

REFERENCES

1. Saeedia P, Petersohna I, Salpea P, et al. *Diab Res Clin Pract* 2019;157: 107843. https://doi.org/10.1016/j.diabres.2019.107843.

2. Ritchie RH, Abel ED. *Circ Res* 2020;126(11): 1501-1525. https://doi.org/10.1161/ CIRCRESAHA.120.315913.

3. Belosludtsev KN, Belosludtseva NV, Dubinin MV. *Int J Mol Sci* 2020;21(18): 6559. https://doi.org/10.3390/ijms21186559.

4. Pinti MV, Fink GK, Hathaway QA, et al. *Am J Physiol Endocrinol Metab* 2019;316(2): E268-E285. https://doi.org/10.1152/ajpendo.00314.2018.

5. Ngo DTM, Sverdlov AL, Karki S, et al. *Am J Physiol Endocrinol Metab* 2019;316(2): E168-E177. https://doi.org/10.1152/ajpen do.00227.2018.

6. Gorbenko NI, Poltorack VV, Gladkih AI, Ivanova OV. *Bull Exp Biol Med* 2001;132: 656-659.

7. Gorbenko NI, Poltorack VV, Ivanova OV. *Diabetologia* 2004;47(Suppl. 1): A278.

8. Gorbenko NI, Poltorack VV, Gladkih AI, Ivanova OV. *Exp Clin Farmacol* 2003;66: 39-42.

9. Skovsø S. *J Diabetes Investig* 2014;5(4): 349-358. https://doi.org/10.1111/jdi.12235.

10. Di Lisa F, Menabò R, Barbato R, Siliprandi N. *Am J Physiol* 1994;267: 455-461._https://doi.org/10.1152/ajpheart.1994.267.2.H455.

11. Staniek K, Nohl H. *Biochim Biophys Acta* 1999;1413(2): 70-80. https://doi.org/10.1016/s0005-2728(99)00083-3.

12. Wang Z, Chen K, Han Y, et al. *J Cardiovasc Pharmacol* 2018;72(6): 259-269. https://doi.org/10.1097/FJC.0000000000000608.

13. Antunes F, Han D, Cadenas E. *Free Radic Biol Med* 2002;33(9): 1260-1267. https://doi.org/10.1016/s0891-5849(02)01016-x.

14. Raza H, John A. *Diabetes Metab Res Rev* 2004;20(1): 72-78._https://doi.org/10.1002/dmrr.422.

15. Sedlak J, Lindsay RH. *Anal Biochem* 1968;25(1): 192-205. https://doi.org/10.1016/0003-2697(68)90092-4.

16. Talbot DA, Brand MD. *Biochim Biophys Acta* 2005; 1709(2): 150-156._https://doi.org/ 10.1016/j.bbabio.2005.07.001.

17. Anastacio MM, Kanter EM, Keith AD, et al. *J Am Coll Surg* 2013;216(6): 1144-1149._https://doi.org/10.1016/j.jamcollsurg.2013.01.048.

18. Straus W. *Biochem Biophys Acta* 1956;19: 58-65. https://doi.org/10.1016/0006-3002(56)90385-7.

19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. *J Biol Chem* 1951;193: 265-275.

20. Malaisse W. *J Diabetologia* 1994;37(Supp2): S36-42. https://doi.org/10.1007/ BF00400824.

21. Saravanan R, Pari L. *J Basic Clin Physiol Pharmacol* 2006;17(2): 115-132. https://doi.org/10.1515/jbcpp.2006.17.2.115.

22. Nguyen T, Nioi P, Pickett CB. *J Biol Chem* 2009;284(20): 13291-13295. https://doi.org/10.1074/jbc.R900010200.

23. Castro L, Tórtora V, Mansilla S, Radi R. *Acc Chem Res* 2019;52(9): 2609-2619. https://doi.org/10.1021/acs.accounts.9b00150.

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Dysfunction of cardiac mitochondria appears to play a substantial role in cardiomyopathy and is a promising therapeutic target for many cardiovascular diseases. Persistent hyperglycaemia and hyperlipidemia are believed to be the main causes of increased oxidative stress, mitochondrial dysfunctions, fibrosis and apoptosis of cardiomyocytes in diabetes. We have previously shown that the low-toxic succinate derivative — Phensuccinal (beta-phenylethylamide-2 hydroxy-succinanylic acid, Phe) possesses antioxidant and anti-inflammatory properties. **The aim** of this study was to assess the effects of Phe on the mitochondrial function and oxidative status in the heart of rats with T2DM.

Materials and Methods. T2DM was induced in Wistar rats by a high-caloric diet during 14 weeks combined with intraperitoneal injections of 25 mg/kg streptozotocin twice per week. All diabetic animals were divided into two groups: treated with vehicle or with Phe (in dose 50 mg/kg/day) for four weeks after diabetes induction. Redox status of rats' heart mitochondria was estimated by determination of reactive oxygen species (ROS) production, GSH level and activity of antioxidant enzymes (Mn-superoxide dismutase, glutathione peroxidase and glutathione reductase). Mitochondrial function was determined by activity of aconitase, succinate dehydrogenase and cytochrome C oxidase in rats' cardiomyocytes.

Results. It was established that Phe inhibited oxidative stress in isolated heart mitochondria of rats with T2DM, which was confirmed by decreasing ROS production and increasing GSH level compared to diabetic rats. The use of Phe led to a normalization of antioxidant enzymes (Mn-superoxide dismutase and glutathione peroxidase) activity in the heart of diabetic rats. In addition, Phe improved metabolic activity of the heart mitochondria activating aconitase and succinate dehydrogenase in cardiomyocytes. We can suggest that Phe ameliorate mitochondrial dysfunction decreasing oxidative stress and preventing deficiency in complex II of ETC in the heart of rats with type 2 diabetes.

Conclusion. The data of the present study confirmed the positive effect of Phe on redox homeostasis and functional state of the heart mitochondria in diabetic rats. We suggest that the use of Phe may contribute to the amelioration of cardiovascular risk in type 2 diabetes.

K e y w o r d s : phensuccinal, type 2 diabetes, functional state of cardiac mitochondria, redox status of the heart mitochondria, rats.

ВПЛИВ ПОХІДНОГО ЯНТАРНОЇ КИСЛОТИ — ФЕНСУКЦИНАЛУ НА МІТОХОНДРІАЛЬНУ ФУНКЦІЮ ТА РЕДОКС-СТАТУС У СЕРЦІ ЩУРІВ ІЗ ЦУКРОВИМ ДІАБЕТОМ 2 ТИПУ

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Відомо, що мітохондріальна дисфункція в серці відіграє суттєву роль у розвитку кардіоміопатії та є перспективною терапевтичною мішенню для багатьох серцево-судинних захворювань. Вважається, що стійка гіперглікемія та гіперліпідемія є основними причинами підвищення оксидативного стресу, мітохондріальної дисфункції, фіброзу та апоптозу кардіоміоцитів при цукровому діабеті (ЦД). Раніше нами було показано, що низькотоксичному похідному янтарної кислоти – фенсукциналу (бета-фенілетіламід-2гідрокси-сукцинанілової кислоти, Ф) притаманні антиоксидантні та протизапальні властивості. **Метою** даного дослідження було визначення впливу фенсукциналу на мітохондріальну функцію і оксидативний статус в серці щурів із цукровим діабетом 2 типу.

Матеріали та методи. ЦД 2 типу індукували у щурів популяції Wistar висококалорійною дієтою протягом 14 тижнів в поєднанні з двома внутрішньочеревними ін'єкціями стрептозотоцину в дозі 25 мг/кг м.т. через тиждень. Діабетичні тварини були розділені на дві групи: щури, які отримували розчинник або Ф (в дозі 50 мг/кг м.т./добу) протягом чотирьох тижнів після індукції діабету. Окислювально-відновлювальний статус мітохондрій серця щурів оцінювали за продукції активних форм кисню (АФК), рівня відновленого глутатіону та активності антиоксидантних ферментів (Mn-супероксиддисмутази, глутатіонпероксидази і глутатіонредуктази). Мітохондріальну функцію визначали за активністю аконітази, сукцинатдегідрогенази та цитохром С оксидази в кардіоміоцитах щурів.

Результати. Встановлено, що Ф гальмує розвиток оксидативного стресу в ізольованих мітохондріях серця щурів із ЦД 2 типу, що підтверджується зниженням продукції АФК та підвищенням рівня відновленого глутатіону в порівнянні з діабетичними тваринами. У той же час, введення Ф супроводжувалося нормалізацією активності антиоксидантних ферментів Mn-супероксиддисмутази і глутатіонпероксидази в серці діабетичних щурів. Крім того, Ф поліпшував метаболічну активність мітохондрій серця, активуючи аконітазу і сукцинатдегідрогеназу в кардіоміоцитах. Ми можемо припустити, що Ф покращує мітохондріальну дисфункцію за рахунок зменшення оксидативного стресу та запобігання дефіциту комплексу II електрон-транспортного ланцюга у серці щурів з ЦД 2 типу.

Висновок. Отримані результати підтвердили позитивний вплив Ф на окислювально-відновлювальний гомеостаз і функціональний стан мітохондрій серця діабетичних щурів, що свідчить про перспективність його використання для зниження ризику серцево-судинних захворювань при ЦД 2 типу.

К л ю ч о в і с л о в а : фенсукцинал, цукровий діабет 2 типу, функціональний стан мітохондрій серця, окислювально-відновний статус мітохондрій серця, щури.