
doi: <https://doi.org/10.15407/dopovidi2018.11.091>

UDC 616.379-008.64-002:577.2

**L.K. Sokolova, V.M. Pushkarev,
Yu.B. Belchina, V.V. Pushkarev, O.V. Furmanova,
S.A. Cherviakova, O.I. Kovzun, M.D. Tronko**

V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine, Kiev

E-mail: pushkarev.vm@gmail.com

NT-proBNP blood levels in patients with type 2 diabetes mellitus with anemia and after the dapagliflozin treatment

Presented by Corresponding Member of the NAS of Ukraine M.D. Tronko

The content of the brain natriuretic peptide (NT-proBNP) in blood plasma and the activity of 5'-adenosine monophosphate-activated protein kinase (AMPK) in lymphocytes of type 2 diabetes patients with anemia and after the treatment with dapagliflozin are determined with enzyme immunoassay. It is shown that the highest level of NT-proBNP is observed in patients with severe anemia. At the treatment with dapagliflozin, the peptide level was markedly reduced, which may be explained by an increase in the activity of AMPK as a result of the exposure to a hypoglycemic drug. It is suggested that the treatment with dapagliflozin may have a positive effect on complications of type 2 diabetes associated with cardiovascular pathologies.

Keywords: *type 2 diabetes, anemia, NT-proBNP, dapagliflozin, AMPK.*

Brain natriuretic peptide (BNP) is used as a marker in the diagnosis of heart failure. ProBNP is released in response to the stimulation of ventricular cardiomyocytes and is split into two fragments: the active hormone BNP (32 amino acids) and the inactive N-terminal peptide NT-proBNP (76 amino acids). BNP is a physiological antagonist of angiotensin II. So, its release and binding to the membrane-bound NPR-A (natriuretic peptide A receptor) mediates natriuresis, vasodilatation, renin inhibition, antimitogenesis, anti-ischaemic effects and positive lusitropism via cGMP signalling. BNP is degraded by the ectoenzyme neutral endopeptidase and cleared by NPR-C receptor, mainly via kidneys [1, 2].

The level of both plasma BNP and NT-proBNP is elevated in patients with left ventricular dysfunction. In this case, the contents of BNP and NT-proBNP in the blood plasma significantly correlate with functional classes of chronic heart failure [3].

NT-proBNP is characterized by a longer half-life, better *in vitro* stability, less biological variability, and higher blood concentrations, than BNP-32. Determining the BNP level in blood

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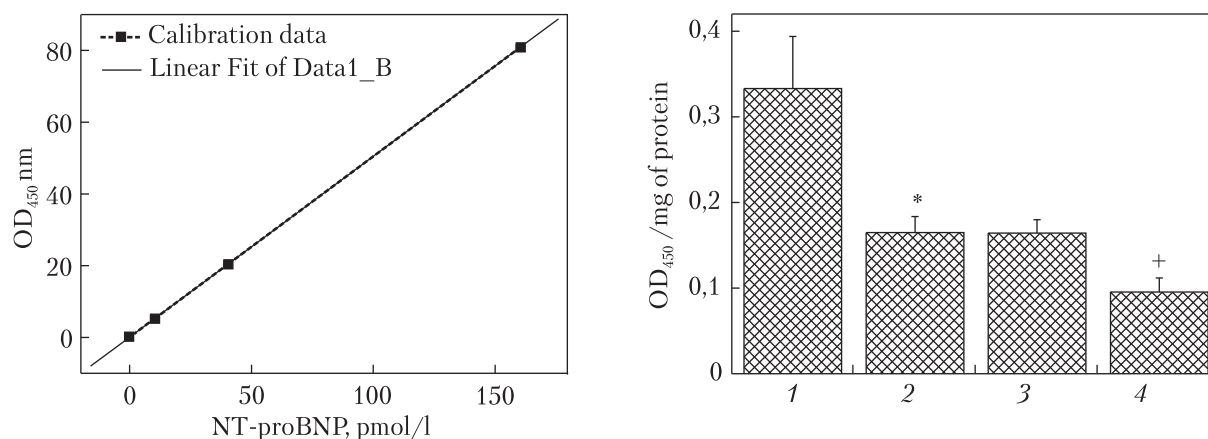


Fig. 1. Calibration curve for the NT-proBNP concentration determination

Fig. 2. Amount of NT-proBNP in the blood of patients with type 2 diabetes. 1 – with severe anemia; 2 – mild anemia; 3 – before the dapagliflozin treatment; 4 – after the dapagliflozin treatment. * – differences between groups 1 and 2 are significant, $P < 0.05$; + – differences between groups 3 and 4 are significant, $P < 0.05$. $M \pm SD$, $n = 6 \div 8$

AMPK activity in patients with diabetes mellitus before and after the treatment with hypoglycemic agents, metformin and dapagliflozin

No.	Groups of patients	Phospho-AMPK, mkg/mg of protein	% to control
1	Control	$0.0178 \pm 0.0039^*$	100
2	T2D before treatment	$0.0095 \pm 0.00048^+$	53.4
3	Metformin	$0.0322 \pm 0.0078^{+*}$	180.7
4	Dapagliflozin	$0.0642 \pm 0.0076^{+*x}$	360.7
5	Metformin + dapagliflozin	$0.0807 \pm 0.0058^{+*x}$	453.4

Notes. Control group (1) comprised individuals who did not have diabetes mellitus, representative by age; 2 – patients with type 2 diabetes before the start of hypoglycemic therapy; 3 – patients with T2D who received generic metformin 1000 mg twice a day as monotherapy; 4 – patients with T2D, receiving dapagliflozin in a daily dose of 10 mg as monotherapy; 5 – patients with T2D on combined therapy (metformin and dapagliflozin).

$M \pm SD$, $n = 2 \div 7$; + – differences from control (1) are significant, $P < 0.05$; * – differences from control (1) and from patients with diabetes before treatment (2) are significant, $P < 0.05$; x – differences from the metformin effects (3) are significant, $P < 0.05$

helps to assess the severity of chronic heart failure, to predict the further development of the disease, and to evaluate the effect of the therapy [1].

Materials and methods. The study was conducted in the diabetology department of the Institute. All patients signed the informed consent to conduct the further diagnostic and research study. Immediately after the collection, blood was diluted in phosphate buffer saline (PBS) 1 : 1, then layered onto 3 ml of Histopaque 1077 (Sigma, USA), centrifuged in a bucket-rotor at 400 g for 30 min. The lymphocytes collected were washed with PBS and frozen at -80°C until use. The cells were lysed in the extraction buffer with inhibitors of proteases and phosphatases.

NT-proBNP was determined using an enzyme-linked immunosorbent assay (ELISA) kit SK-1204 (Biomedica, Austria). To determine the amount of phospho-AMPK (phospho-threonine 172), an ELISA kit ab154468 (Abcam, UK) was used. The studies were carried out in triplets. The protein concentration in blood plasma and cell lysate was determined using a Novagen (USA) BCA protein assay kit. The measurements were carried out on a microplate reader (Biotek Instruments, USA) at a wavelength of 450 (NT-proBNP) or 600 (AMPK) nm.

To get the calibration curve for the AMPK determination (not shown), a kidney cell culture HEK293T of human embryonic kidney was used, which is recommended by the manufacturer as a positive control.

The calibration curve for the NT-proBNP determination perfectly coincides with the theoretical line, which indicates no scattering of data (Fig. 1).

The results of studies are presented as $M \pm SD$, $n = 2 \div 8$. To compare the data groups, Student's *t*-test was used. Values of $P \leq 0.05$ were considered as significant.

Results and their discussion. As can be seen from Fig. 2, the highest level of NT-proBNP is observed in the blood of patients with type 2 diabetes (T2D) and severe anemia. With mild anemia, the amount of peptide is more than two times lower. In the dapagliflozin-treated patients with type 2 diabetes, the levels of NT-proBNP were additionally lower by 42 %.

Anemia is defined as a reduction of the hemoglobin concentration in blood, which consequently reduces the oxygen-carrying capacity of red blood cells. Anemia of chronic diseases such as diabetes, cancer, renal and heart failures is the most common cause for low hemoglobin [4, 5]. Anemia is commonly observed in subjects with diabetes mellitus. It was demonstrated that anemia is linked to an increased risk of a hypoxia-induced organ damage including cardiovascular events and mortality [6].

Dapagliflozin is a powerful (inhibition constant of 0.55 nM), selective, and reversible inhibitor of the sodium/glucose cotransporter 2 (SGLT2) responsible for the reabsorption of glucose in the proximal tubules of kidney [7].

AMPK controls the energy balance of cells. With type 2 diabetes and obesity, its activity decreases, and the activity of protein kinases mTORC1/p70S6K increases, leading to the phosphorylation of insulin receptor substrates and insulin resistance [8, 9]. It has been shown that the activation of AMPK inhibits the development of cardiac hypertrophy through pathways that involve eukaryotic elongation factor-2 (eEF2), p70S6 kinase (p70S6K), and mammalian target of rapamycin (mTOR) [10].

Dapagliflozin itself significantly increases the activity of AMPK (Table) and, moreover, enhances the effect of generic metformin. Thus, a decrease of the BNP concentration in blood may be associated with a stimulation of the AMPK activity by dapagliflozin and indicates a possible improvement in the cardiomyocytes function. As was also shown earlier, an increase in HIF1 expression, AMPK activity, and ATP consumption and a suppression of apoptosis in epithelial cells may indicate additional positive effects of dapagliflozin in cardiovascular diseases [11].

Conclusions. Patients with type 2 diabetes and severe anemia are characterized by a high level of NT-proBNP in blood.

Dapagliflozin-treated patients had a lower level of NT-proBNP that can be explained by the effect of the drug on the AMPK activity.

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Received 22.06.2018

*Л.К. Соколова, В.М. Пушкарёв,
Ю.Б. Бельчина, В.В. Пушкарёв, О.В. Фурманова,
С.А. Червякова, О.І. Ковзун, М.Д. Тронько*

ДУ "Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України", Київ
E-mail: pushkarev.vm@gmail.com

**ВМІСТ NT-proBNP У КРОВІ ПАЦІЄНТІВ З ЦУКРОВИМ ДІАБЕТОМ
2-го ТИПУ З АНЕМІЄЮ І ПІСЛЯ ЛІКУВАННЯ ДАПАГЛІФЛОЗИНОМ**

Методом імуноферментного аналізу визначено вміст мозкового натрійуретичного пептиду (NT-proBNP) у плазмі крові та активність 5'-аденозинмонофосфатактивованої протеїнкінази (АМПК) в лімфоцитах хворих на діабет 2-го типу з анемією і при лікуванні дапагліфлозином. Показано, що найвищий рівень NT-proBNP спостерігається у хворих з важкою формою анемії. При лікуванні дапагліфлозином рівень пептиду помітно знижувався, що, можливо, пояснюється підвищенням активності АМПК у результаті дії

гіпоглікемічного препарату. Зроблено припущення, що лікування дапагліфлозином може мати позитивний ефект при ускладненнях діабету 2-го типу, пов'язаних із серцево-судинними патологіями.

Ключові слова: діабет 2-го типу, анемія, NT-proBNP, дапагліфлозин, АМРК.

Л.К. Соколова, В.М. Пушкарев,

Ю.Б. Бельчина, В.В. Пушкарев, О.В. Фурманова,

С.А. Червякова, Е.И. Ковзун, Н.Д. Трощко

ГУ "Інститут ендокринології і обміну речовин ім. В.П. Комиссаренко НАМН України", Київ

E-mail: pushkarev.vm@gmail.com

СОДЕРЖАНИЕ NT-proBNP В КРОВИ ПАЦИЕНТОВ С САХАРНЫМ ДИАБЕТОМ 2-го ТИПА С АНЕМИЕЙ И ПОСЛЕ ЛЕЧЕНИЯ ДАПАГЛИФЛОЗИНОМ

Методом иммуноферментного анализа определяли содержание мозгового натрийуретического пептида (NT-proBNP) в плазме крови и активность 5'-аденозинмонофосфатактивируемой протеинкиназы (АМРК) в лимфоцитах больных диабетом 2-го типа с анемией и при лечении дапагліфлозином. Показано, что наиболее высокий уровень NT-proBNP наблюдается у больных с тяжелой формой анемии. При лечении дапагліфлозином уровень пептида заметно снижался, что, возможно, объясняется повышением активности АМРК в результате воздействия гипоглікемического препарата. Сделано предположение, что лечение дапагліфлозином может оказывать положительный эффект при осложнениях диабета 2-го типа, связанных с сердечно-сосудистыми патологиями.

Ключевые слова: диабет 2-го типа, анемия, NT-proBNP, дапагліфлозин, АМРК.