The level of endothelin-1 in the blood of patients with diabetes, treated with hypoglycemic drugs

L.K. Sokolova, Y.B. Belchina, V.V. Pushkarev, S.A. Cherviakova, T.S. Vatseba, O.I. Kovzun, V.M. Pushkarev

Abstract. Background. Endothelin (ET) is one of the most significant regulators of the functional state of vascular endothelium. ET-1 is the principal cardiovascular isoform of the endothelin system. ET-1 has both inflammatory and proliferative affects and contributes to pathogenic processes in the cardiovascular system. In diabetes, the rise of glucose concentration effects the formation of ET-1. The aim of the work was to study the content of ET-1 in the blood of patients with diabetes treated with various hypoglycemic agents. Material and methods. The amount of ET-1 was evaluated via ELISA in 103 individuals: 17 healthy volunteers and 86 patients with diabetes. To determine the concentration of ET-1 the endothelin (1-21) EIA kit (Biomedica) was used. Glycated hemoglobin was determined using one HbA1c FS kit — DiaSys Diagnostic Systems. Results. The average level of endothelin in the blood of patients with diabetes was 0.536±0.047 fmol/ml vs. control — 0.118±0.017 fmol/ml. We did not observed changes of ET-1 levels during monotherapy with metformin or insulin. At metformin + insulin + dapagliflozin, insulin + metformin and sulfonylurea + metformin but not DPP-4 inhibitor + metformin combinations (T2D) there was decrease of ET-1 level in the blood. It is of interest that DPP-4 inhibitor + metformin caused significant increase (0.767±0.043 fmol/ml) of ET-1 concentration. Conclusion. Thus, with combination therapy, except pair DPP-4 inhibitor + metformin, the level of ET-1 in the blood of patients with diabetes decreased significantly more than with monotherapy. Keywords: diabetes, endothelin-1, metformin, insulin, dapagliflozin, sulfonylurea, DPP-4 inhibitor.

Introduction

Diabetes mellitus is a group of metabolic disorders with high blood glucose levels over a prolonged period. Patients with diabetes are associated with endothelial dysfunction (ED), which is a key pathological event in the development of chronic diabetic complications [1]. ED is a systemic pathological state of the endothelium that is defined as an imbalance between vasodilating and vasoconstricting substances produced by the endothelium. An important effect of ED is that it leads to increased production and
biological activity of the potent vasoconstrictor and pro-inflammatory peptide endothelin (ET). Diabetes is one of diseases associated with pathologically elevated levels of ET [2].

ET-1 is the principal cardiovascular isoform of the endothelin system. Vascular ET-1 is produced primarily in the endothelium, although it can also be produced in vascular smooth muscle cells (VSMC), macrophages, leukocytes, cardiomyocytes, and fibroblasts. Several mechanisms are involved in the clearance of ET-1 from plasma, including endocytosis in the lungs, enzymatic degradation, degradation of the endothelin B-receptor-ligand complex, and enzymatic processes in the kidney and liver [3, 4]. Two receptor subtypes, endothelin A- and B-receptors (ETA and ETB), mediate the effects of ET-1. VSMC express both ETA and ETB, while endothelial cells express primarily ETB. On smooth muscle cells, ETA mediates vasoconstriction and mitogenesis, while ETB receptor has a dual function and has been shown to cause both vasoconstriction and vasodilation [5]. Alteration in ET-1 balance of the endothelium is the key event in the initiation of arteriosclerosis [6].

The aim of the work was to study the content of ET-1 in the blood of patients with diabetes treated with various hypoglycemic agents.

Materials and methods

The level of ET-1 was evaluated by ELISA in 103 individuals: 17 healthy volunteers and 86 patients with diabetes. Most patients had type 2 diabetes (T2D). The study protocol was approved by the Institute’s ethics committee. All participants provided written informed consent to the use of their biomaterials for further research and diagnostics. Blood was obtained via standard venipuncture and stored in EDTA vacutainer tubes. Plasma was separated via centrifugation within 10 min. after blood sampling. The samples were stored at –80 °C until use. To determine the concentration of ET-1 the endothelin (1-21) EIA kit, Biomedica (Austria) was used. The measurement was carried out at an optical wavelength of 450 nm. Glycated hemoglobin was determined using one HbA1c FS kit — DiaSys Diagnostic Systems GmbH (Germany). The measurement was carried out at an optical wavelength of 660 nm.

Statistical calculations and data presentation were performed using Origin 7.0 software. The results of the study are presented as M±Std. To compare the data groups, Student’s t-test and one-way ANOVA were used. Values of p≤0.05 were considered as significant.

Results

All patients with diabetes had blood ET-1 level higher than the control group (Table 1). The average concentration of ET-1 in the blood of patients with diabetes (n=86) was 0.536±0.047 fmol/ml (control — 0.118±0.017 fmol/ml; n=17). The average level of glycated hemoglobin in the blood of patients with diabetes was 8.64±0.19%. Differences in the amount of ET-1 in the blood of patients between the 1st and 2nd types of diabetes were insignificant (not shown).

Monotherapy with metformin did not cause any changes in ET-1 content compared to the average level (Table 1, lines 2, 3). Insulin monotherapy in type 1 diabetes (T1D) patients also did not affect ET-1 level (Table 1, line 4). Combination therapy with metformin and insulin in T2D patients leads to significant decrease of ET-1 concentration in the blood compared to average diabetic level, as well as metformin and insulin monotherapy. Another combination:

<table>
<thead>
<tr>
<th>N</th>
<th>Therapy</th>
<th>ET-1 (fmol/ml)</th>
<th>Std ± n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.118</td>
<td>0.017</td>
</tr>
<tr>
<td>2</td>
<td>Average T1D/T2D</td>
<td>0.536</td>
<td>0.047</td>
</tr>
<tr>
<td>3</td>
<td>Metformin, T2D</td>
<td>0.470</td>
<td>0.047</td>
</tr>
<tr>
<td>4</td>
<td>Insulin, T1D</td>
<td>0.648</td>
<td>0.096</td>
</tr>
<tr>
<td>5</td>
<td>Insulin + metformin, T2D</td>
<td>0.307</td>
<td>0.044</td>
</tr>
<tr>
<td>6</td>
<td>Sulfonylurea + metformin, T2D</td>
<td>0.352</td>
<td>0.046</td>
</tr>
<tr>
<td>7</td>
<td>Sulfonylurea + metformin + DPP-4 inh., T2D</td>
<td>0.511</td>
<td>0.088</td>
</tr>
<tr>
<td>8</td>
<td>DPP-4 inh. + metformin, T2D</td>
<td>0.767</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Note. Differences from the control are significant (p<0.05) for all diabetic patients; the numbers indicate significant differences from the corresponding group, (p<0.05).

Insulin monotherapy in type 1 diabetes (T1D) patients also did not affect ET-1 level (Table 1, line 4). Combination therapy with metformin and insulin in T2D patients leads to significant decrease of ET-1 concentration in the blood compared to average diabetic level, as well as metformin and insulin monotherapy. Another combination:
metformin + sulfonylurea also caused significant decrease of ET-1 level in the patients’ blood. But the same combination with dipeptidyl peptidase-4 (DPP-4) inhibitors therapy increased level of ET-1 to average values (Table 1, line 7), and metformin + dipeptidyl peptidase-4 (DPP-4) inhibitors therapy significantly increased the amount of ET-1 to up to 0.767±0.043 fmol/ml, which is higher than the average level.

Important indicators for T2D are the duration of the disease, body mass index (BMI) and the Hb1Ac level in the blood. Another group of patients was analyzed taking into account these indicators. The data obtained show that combined treatment much more effectively reduces ET-1, even though the duration of the disease and the concentration of Hb1Ac with monotherapy are significantly lower than with combined therapy (Table 2). The most effective was the combination of metformin and insulin with SGLT2-inhibitors (dapagliflozin), at which ET-1 content decreased by 2 times compared with the average level (Table 2, line 3).

<table>
<thead>
<tr>
<th>Therapy</th>
<th>ET-1</th>
<th>BMI</th>
<th>Duration of diabetes, years</th>
<th>Hb1Ac, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Metformin</td>
<td>0,489</td>
<td>32,083</td>
<td>7,727</td>
<td>7,429</td>
</tr>
<tr>
<td>Std</td>
<td>0,100</td>
<td>1,336</td>
<td>1,941</td>
<td>0,423</td>
</tr>
<tr>
<td>2. Metformin + insulin</td>
<td>0,303*</td>
<td>32,797</td>
<td>18,211*</td>
<td>9,287*</td>
</tr>
<tr>
<td>Std</td>
<td>0,041</td>
<td>1,530</td>
<td>2,864</td>
<td>0,433</td>
</tr>
<tr>
<td>3. Metformin + insulin + SGLT2 inhib.</td>
<td>0,267*</td>
<td>32,862</td>
<td>16,167*</td>
<td>9,760*</td>
</tr>
</tbody>
</table>

Note. * — differences from group 1 are significant (p<0.05).

Thus, with combination therapy, the level of ET-1 in the blood of patients with diabetes decreased significantly compared to monotherapy. Metformin + insulin + dapagliflozin and insulin + metformin combinations (both at T2D) were the most effective, concerning decrease of ET-1 level in the blood.

**Discussion**

The molecular mechanisms of pathological factors and therapeutic agents (hypoglycemic drugs) influence on ET-1 secretion in diabetes are not well studied. ET-1 is synthesized and released continuously from endothelial cells. Synthesis of the biologically active ET-1 is a multistep process, and regulatory mechanisms obviously exist on each of these post-translational processing steps, however, transcriptional regulation is thought to be the major mechanism controlling ET-1 bioavailability. ET-1 localizes to both constitutive secretory vesicles and specialized regulatory granules — Weibel-Palade bodies in endothelial cells. Some agents enhance the ET-1 content via exocytosis of Weibel-Palade bodies but also stimulate edn1 mRNA levels [7].

Levels of preproET-1 are modulated predominantly at the level of transcription, with implicating numerous transcription factors including activator protein 1 (AP-1), nuclear factor kappa B (NF-κB), FOXO1 (forkhead box protein O1), VezF1 (vascular endothelial zinc finger 1), HIF-1 (hypoxia-inducible factor 1), and GATA2. One of the most important regulators of ET-1 production in endothelial cells is transforming growth factor (TGF-β) [8].

The effect of insulin on ET-1 expression can be determined by various mechanisms. Through PI3K, Akt phosphorylation and inhibition of GSK-3, insulin derepresses VezF1, stimulating the expression of edn1. In addition, through PKC it can activate AP-1 and edn1 mRNA synthesis. Finally, excess glucose in diabetes stimulates the recruitment of NF-κB and p300 to the edn1 promoter and binding of these factors is associated with an increase in histone H3 acetylation and ET-1 expression [9]. The activation of these mechanisms should result in enhanced ET synthesis, which we have not observed (Table 1, line 4). Obviously, there are restraining mechanisms and one of them is possibly an insulin-dependent decrease in glucose and Hb1Ac levels and respectively inhibiting of NF-κB-directed edn1 expression. In addition, there is evidence that ET-1 suppressed insulin-induced Akt phosphorylation (activation) at both Thr308 and Ser473 residues [10], which can lead to repression of VezF1 and, accordingly, a decrease in ET synthesis — a feedback mechanism.

Metformin per se also did not have a noticeable effect on ET levels (Table 1, 2), although a positive effect of the drug on endothelial function in diabetes has been reported. So, metformin positively affects the viability and functions of...
the endothelium, it has also been found to markedly improve endothelial-dependent vasodilation, and simultaneously reduce the expression of dysfunctional biomarkers such as endothelin-1 (ET-1), plasminogen activator inhibitor-1 (PAI-1), and C-Reactive protein (CRP) in endothelial cells. There are data that metformin antagonizes the proliferation and migration of human aortal smooth muscle cells through activation of AMPK [11]. It has been hypothesized that inhibition of the NF-κB by metformin [1, 12] down-regulates the inflammatory response, and this might be the key answer to the reduction in the cardiovascular events. However, other studies have challenged this hypothesis by demonstrating minimal, or no influence of metformin on the inflammatory processes [13].

In combination therapy with insulin and metformin, the ET-1 level in patients with T2D was significantly reduced. This is difficult to explain given the complex relationships of both agents. Metformin therapy improves insulin secretion and protects against pancreatic β-cell apoptosis [14]. Metformin-mediated activation of AMPK inhibits the NF-κB cascade, resulting in improved insulin signaling [1, 12]. However, insulin suppresses AMPK activity in leucocytes, liver, muscles, and possibly in other tissues and organs [15, 16]. There is a certain independence of the insulin signal pathway (PI3K/Akt) from the cascade of AMPK activation (LKB1, CAMKK2, and TAK1 (TGF-β-activated kinase 1).

TGF-β, which is involved in the pathogenesis of numerous diseases, and is important regulator of ET-1 production in endothelial cells [8, 17], is target of metformin. The direct binding of metformin to TGF-β1 was identified that inhibits growth factor binding to its receptor [18], and probably can reduce ET-1 synthesis.

Combinations of metformin + sulfonylurea and metformin + insulin + dapagliflozin also caused significant decrease of ET-1 level in the blood. Sulfonylurea treatment also downregulated TGF-β in the blood of patients with T2D [17] and in db/db mice [19], which could be the reason of ET-1 decrease. Compelling evidence has been obtained showing that dapagliflozin significantly improves endothelial function [20-23]. It was demonstrated that dapagliflozin-mediated attenuation of TNFα- and hyperglycaemia-induced increases in intercellular adhesion molecule-1, vascular cell adhesion molecule-1, PAI-1 and NF-κB expression [24].

The most interesting is increased level of ET-1 with DPP-4 inhibitors therapy. Obtained data suggest that DPP-4 inhibitors exert favourable effects on the vascular endothelium. They suppress the degradation of glucagon-like peptide-1 (GLP-1), which can enhance NO production. The drug inhibits ET-1 expression in the aortic endothelium by suppressing the NF-κB/1κBα system through the activation of the AMPK pathway in diabetic rats [25]. On the other hands in contrast to the use of SGLT-2 inhibitors or GLP-1 agonists, which were associated with reduced mortality, use of DPP-4 inhibitors did not result in lower mortality of participants with T2D [20]. Moreover, the FDA has issued a warning that medications for T2D containing saxagliptin and alogliptin may increase the risk for heart failure, particularly in patients with cardiovascular disease or renal impairment [26]. Perhaps an increase in ET in the treatment of DPP-4 inhibitors, which we observed, is somehow related to the negative consequences of such treatment.

**Conclusion**

Thus, in contrast to monotherapy, a combination therapy with hypoglycemic drugs, led to a significant decrease in ET-1 levels in the diabetic patients’ blood. The most effective was the combination of metformin, insulin and dapagliflozin, which could possibly be recommended for patients with T2D and cardiovascular diseases.

**References**


Оригінальні дослідження

ня (0,767±0,043 фмоль/мл) концентрації ET-1. Висновки. Отже, на тлі комбінованої терапії, за винятком пари інгібітор DPP-4 + метформін, рівень ET-1 у крові пацієнтів із діабетом істотно знижувався, на відміну від монотерапії.

Ключові слова: діабет, ендотелін-1, метформін, інсулін, дапагліфлозин, сульфанілмочевина, інгібітор DPP-4.

Уровень ендотеліна-1 в крові больных диабетом при терапии сахароснижающими препаратами

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Резюме. Актуальность. Эндотелин (ЕТ) является одним из наиболее значимых регуляторов функционального состояния эндотелия сосудов. ET-1 является основной сердечно-сосудистой изоформой системы эндотелия. ET-1 оказывает как воспалительное, так и пролиферативное действие и способствует патогенным процессам в сердечно-сосудистой системе. При диабете повышение концентрации глюкозы влияет на образование ET-1. Цель. Целью работы было изучение содержания ET-1 в крови пациентов с сахарным диабетом, получавших различные гипогликемические препараты. Материал и методы. Количество ET-1 оценивали с помощью ИФА у 103 человек: 17 здоровых добровольцев и 86 пациентов с диабетом. Для определения концентрации ET-1 использовали набор ЕIA для эндотелина (1-21) (Biomedica). Гликированный гемоглобин определяли с использованием набора one HbA1c FS — DiaSys Diagnostic Systems. Результаты. Средний уровень ET в крови больных сахарным диабетом составил 0,536±0,047 фмоль/мл, в контроле — 0,118±0,017 фмоль/мл. Мы не наблюдали изменений уровней ET-1 при монотерапии метформином или инсулином. При комбинациях метформин + инсулин + дапагліфлозин, инсулин + метформин и сульфанилмочевина + метформин, но не в комбинации ингибитор DPP-4 + метформин (СД2) наблюдалось снижение уровня ET-1 в крови. Интересно, что ингибитор DPP-4 + метформин вызывали существенное увеличение (0,767±0,043 фмоль/мл) концентрации ET-1. Выводы. Таким образом, при комбинированной терапии, за исключением пары ингибитор DPP-4 + метформин, уровень ET-1 в крови пациентов с диабетом значительно снижался, в отличие от монотерапии.

Ключевые слова: диабет, эндотелин-1, метформин, инсулин, дапаглифлозин, сульфанилмочевина, ингибитор DPP-4.


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Contribution: L.K. Sokolova, V.M. Pushkarev — Project idea, data analysis, paper writing, Y.B. Belchina, S.A. Cherviakova, T.S. Vatseba — Biomaterial sampling, primary analysis, case management, V.V. Pushkarev — Carrying out an experiment, preparing an paper for publication, translation, O.I. Kovzun — Editing an article.

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