The role of plasma endothelin-1 in diabetes mellitus

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Introduction

The endothelium, a monolayer lining the blood vessels, has several important homeostatic roles. Endothelial cells participate in blood coagulation and fibrinolysis; platelet, leukocyte cells and lipoprotein interactions with the vessel wall, they regulate vascular tone, the blood pressure and smooth muscle cell proliferation. Endothelial cells produce various endogenous factors - prostacyclin, nitric oxide, angiotensin, endothelium derived hyperpolarizing factor, free radicals, bradykinin, thromboxane, endothelin-1 -, which act partly locally in autocrin - paracrin fashion as well as hormonal factors, acting further afield from their place of production. The system is vulnerable because of its complexity and the balance may be disturbed by numerous endogenous and exogenous agents.

In diabetes, vascular disease is the leading cause of morbidity and mortality. Early diagnosis and therapy of vascular damage is essential to the survival of diabetic patients.

Finding a factor that could easily be detected and measured in the plasma of diabetic patients, and at concentrations which would indicate endothelial dysfunction at the earliest phase would have an enormous impact in diabetes.

Endothelin-1, produced by vascular endothelial cells, is the most potent vasoconstrictor factor known with tissue proliferative properties. Several studies have shown that ET-1 release increases in endothelial dysfunction. ET-1 elevation may be related to increased incidence and severity of vascular disease in diabetes mellitus.

Aims

The subject of our study was to investigate the role of plasma endothelin-1 in diabetes mellitus. The data of 239 subjects has been processed during clinical trials. Type 1 and type 2 diabetic patients, healthy control subjects, hypertensive non-diabetic patients and hyperlipidemic non-diabetic patients were included in the studies.

Our aims were

- to investigate whether plasma ET-1 concentration changes in diabetic patients with and without vascular complication,
- to study if changes in plasma ET-1 levels indicate the severity of vascular complications,
- to evaluate whether hypertension associated with diabetes influences the plasma concentration of ET-1,
- to investigate whether changes in plasma ET-1 concentration are an early marker of vascular damage,
to study the effect of hyperlipidemia on plasma ET-1 concentration in diabetes.

to evaluate if there is an association between plasma ET-1 concentration, plasma glucose and HbA1c level.

to investigate the effect of oral antidiabetic drugs and insulin therapy on plasma ET-1 concentration and to study whether there is a relationship between endogenous insulin production and plasma ET-1 level.

In type 2 diabetic patients our aim was to study endothelial dysfunction. Beside plasma ET-1 concentration we measured plasma total nitrite + nitrate (NO₂+NO₃) levels which are an indirect marker of the vasodilator nitric oxide production.

Our aim was to investigate

- whether there is a link between plasma concentration of total NO₂+NO₃ and plasma ET-1.
- how hypertension influences the plasma level of total NO₂+NO₃.
- if there is a link between plasma total NO₂+NO₃ concentration and the method of treatment in type 2 diabetes mellitus.

We have investigated how the different extraction methods used during ET-1 radioimmunoassays influence the sensitivity of the assay.

**Methods**

**Measurement of circulating plasma ET-1 concentration**

Plasma samples in case of type 1 diabetic patients were extracted based on acetone, In type 2 diabetic patients, samples were extracted using Sep-Pak C18 chromatographic columns (Waters, Millipore). Supernatants were separated and dried by Speedvac concentrator, then reconstituted in RIA buffer. Extracted samples were measured by radioimmunoassay based on Peninsula antibody. The sensitivity of the ET-1 assay was 0,3 fmol/tube.

**Plasma total nitrite + nitrate measurement**: the basal end products of NO metabolism [nitrite + nitrate (NO₂+NO₃)] in the plasma were measured colorimetrically using Griess reagent after reducing nitrate to nitrite with cadmium beads (samples were incubated on room temperature for 24 hours). Samples were measured by ELISA reader, wavelength: 540nm (OXIS International Inc, BIOTECH, Nitric oxide Non-enzymatic Assay, Cat. No: 22111N)
Results:

A. Type 1 diabetes mellitus
1. In hyperlipidemic diabetic patients plasma ET-1 concentration was significantly higher compared to control subjects (p<0.05).
2. Presence of diabetic complications in the hyperlipidemic diabetic group had further increased plasma ET-1 concentration and was significantly higher than in normolipidemic diabetic patients, in hyperlipidemic non-diabetic patients and in control subjects (p<0.001).
3. There was no difference in plasma ET-1 concentration between normolipidemic diabetic patients without complications, hyperlipidemic non-diabetic patients and control subjects.
4. Studying the effect of late diabetic complications we have found significantly higher plasma ET-1 level in type 1 diabetic patients suffering from late diabetic complications than in control subjects and in diabetic patients without complication (p<0.01).
5. Investigating the marker function of plasma ET-1 level, we showed that elevated plasma ET-1 concentration could already be detected in type 1 diabetic patients where no diabetic complication could be diagnosed with the exception of hypertension.
6. There was no difference in plasma ET-1 concentration between control subjects and type 1 diabetic patients without symptoms of diabetic complications.
7. No correlation was found in type 1 diabetes mellitus between plasma ET-1 concentration and plasma HbA1c, serum glucose, serum creatinin level, serum apo A1, apo B level, plasma C-peptide and serum insulin concentration, the time of diabetes, body mass index, and age.
8. There was no correlation between daily exogenous insulin treatment and plasma ET-1 level.

In type 1 diabetic patients plasma total antioxidant activity (TAS) was also measured.
1. We have found no correlation between total antioxidant activity and plasma ET-1 concentration.
2. We have found no association between plasma TAS and plasma ET-1 concentration between hyper- and normolipidemic type 1 diabetic patients, hyperlipidemic non-diabetic patients and control subjects.

B. Type 2 diabetes mellitus
1. We have found significantly higher plasma ET-1 concentration in type 2 diabetic patients suffering from ischaemic organ damage than in patients with hypertension alone.
2. Studying endothelial dysfunction we have found **significantly lower plasma total nitrite + nitrate** \((\text{NO}_2+\text{NO}_3)\) level in hypertensive type 2 diabetic patients compared to normotensive diabetic patients. Plasma ET-1 concentration did not differ between the two groups and the control group.

3. There **was no difference** in plasma ET-1 levels between type 2 diabetic patients and control subjects.

4. Plasma ET-1 concentration was **similar** in normo- and hyperlipidemic type 2 diabetic patients, in hyperlipidemic non-diabetic patients and control subjects.

5. There was **no difference** in plasma ET-1 concentration between diabetic patients with and without diabetic complications, and control subjects.

6. Plasma total \(\text{NO}_2+\text{NO}_3\) concentration was **similar** in control subjects and type 2 diabetic patients.

7. There was **no difference** in plasma ET-1 concentration and plasma total \(\text{NO}_2+\text{NO}_3\) between patients treated with antidiabetic drugs and those on insulin therapy.

8. **No correlation was found between plasma ET-1 concentration** and plasma HbA1c, serum glucose, serum creatinin level, plasma \(\text{NO}_2+\text{NO}_3\) and plasma C-peptide concentration, body mass index, and age in type 2 diabetes mellitus.

9. **No association was found between plasma total NO\(_2\)+NO\(_3\) concentration** and plasma C-peptide, plasma HbA1c level, serum glucose concentration, age and body mass index in type 2 diabetic patients.

**C.** We have investigated how **different extraction methods used during ET-1 radioimmunoassays** influence the sensitivity of the assay.

We have found a strong positive correlation between the extraction method based on acetone and the one using Sep-Pak C18 chromatographic columns, with the latter proving to be more sensitive.

**Conclusions**

1. Our results prove that in **type 1 diabetes mellitus**

   - elevation of plasma ET-1 concentration is an early marker of vascular disease.
• elevated plasma ET-1 concentration detected in hyperlipidemic diabetic patients is a possible marker of endothelial dysfunction and indicates vascular damage in an early phase – before clinically manifest symptoms appear.

Endothelin-1 – considering its complex effects – can be one of the initiating factors of the development of accelerated vascular disease in diabetes mellitus. Presence of hyperlipidemia causes a further acceleration of the atherogenic process.

2. In type 2 diabetes mellitus
• plasma ET-1 concentration is not an early and sensitive marker of vascular disease.
• elevated plasma ET-1 level may indicate the presence of advanced but yet clinically silent ischaemic organ damage, therefore when plasma ET-1 level is increased - because of the risk of silent ischaemia - cardiovascular check-up is recommended.
• lower plasma NO₂+NO₃ found in hypertensive diabetic patents might be a sign of the development of endothelial dysfunction.

3. There was no correlation between plasma ET-1 concentration and plasma HbA1c, serum glucose, serum creatinin level, body mass index and age in type 1 and type 2 diabetes mellitus.

4. Comparing the different extraction methods, extraction with Sep-pack C18 chromatographic column has proven to be the most sensitive.

The difference in the results of type 1 and type 2 diabetic patients might be due to the different pathomechanism of the two diseases. In type 1 diabetes mellitus increased concentration of ET-1 proved to be an early and sensitive marker of endothelial dysfunction however the same could not be proved in type 2 diabetes mellitus. In type 2 diabetes endogenous hyperinsulinaemia occurs. Insulin stimulates ET-1 production and secretion, but presumably a larger portion of ET-1 is released abluminally, therefore the plasma concentration of ET-1 does not necessarily reflect the increased quantity secreted toward smooth muscle cells. Insulin also raises the number of ET₃ receptors, therefore ET-1 absorption and its effects can increase despite the fact that this is not reflected in the plasma. Measuring tissue ET-1 concentration would be more appropriate in type 2 diabetes however this is difficult to carry out in vivo.
List of original papers used in the thesis


2. B. Sármán, M. Tóth, A. Somogyi: Role of Endothelin-1 in Diabetes Mellitus. Diabetes/Metabolism Reviews, 14: 171-175, 1998. (IF: 1,955)


5. B. Sármán, K. Farkas, M.Tóth, A. Somogyi: Endothelin-1 is a marker of advanced vascular complications in type 2 diabetes mellitus. (handed in for publication)


Other publications


ABSTRACTS


19. Farkas K, **Sármán B**, Molnár J, Kocsis I, **Somogyi A**: Antioxidant vitamin status in IDDM and hyperlipidaemia. Diabetologia 41, Suppl. 1, A349, 1998.(**IF: 4,986**)
