Accumulation of fructosyl-lysine and advanced glycation end products in the kidney, retina and peripheral nerve of streptozotocin-induced diabetic rats

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Abstract

The accumulation of AGEs (advanced glycation end products) in diabetes mellitus has been implicated in the biochemical dysfunction associated with the chronic development of microvascular complications of diabetes – nephropathy, retinopathy and peripheral neuropathy. We investigated the concentrations of fructosyl-lysine and AGE residues in protein extracts of renal glomeruli, retina, peripheral nerve and plasma protein of streptozotocin-induced diabetic rats and normal healthy controls. Glycation adducts were determined by LC with tandem MS detection. In diabetic rats, the fructosyl-lysine concentration was increased markedly in glomeruli, retina, sciatic nerve and plasma protein. The concentrations of Nε-carboxymethyl-lysine and Nε-carboxyethyl-lysine were increased in glomeruli, sciatic nerve and plasma protein, and Nδ-carboxymethyl-lysine also in the retina. Hydroimidazolone AGEs derived from glyoxal, methylglyoxal and 3-deoxyglucosone were major AGEs quantitatively. They were increased in the retina, nerve, glomeruli and plasma protein. AGE accumulation in renal glomeruli, retina, peripheral nerve and plasma proteins is consistent with a role for AGEs in the development of nephropathy, retinopathy and peripheral neuropathy in diabetes. High-dose therapy with thiamine and Benfotiamine suppressed the accumulation of AGEs, and is a novel approach to preventing the development of diabetic complications.

Introduction

Microvascular disease (nephropathy, retinopathy and neuropathy) develops in human subjects with diabetes mellitus over 10–15 years. It is a common and disabling complication of diabetes mellitus, with no effective therapy. Diabetic nephropathy is characterized by the development of proteinuria, culminating in end-stage renal disease with a particularly high risk of cardiovascular morbidity and mortality. The initial stage of development of nephropathy, incipient nephropathy, is characterized by the onset of persistent microalbuminuria and hyperfiltration [1]. Diabetic retinopathy is characterized by early loss of pericytes, vessel weakening and endothelial dysfunction that leads to the development of acellular capillaries, microaneurysms, capillary closure and ischaemia. In the later stages, proliferative retinopathy, there is angiogenesis, macular oedema and severe visual impairment [2]. Diabetic neuropathy is a spectrum of damage to peripheral nerves in diabetes mellitus. It arises as a result of progressive damage to the peripheral sensory and autonomic nervous systems [3]. Hyperglycaemia is a risk factor for the development of microvascular complications in both Type I and Type II diabetic subjects [4,5]. Tight control of blood glucose (and blood pressure) decreases the risk of developing microvascular complications, but this is not always achievable because of limitations of current drug therapy [6].

The link between hyperglycaemia and the development of microvascular complications is explained by the effect of plasma glucose concentration on vascular cells with GLUT1 glucose transporter expression. A high plasma glucose concentration leads to a high cytosolic glucose concentration in capillary endothelial cells and pericytes, with consequent biochemical dysfunction, including increased formation and accumulation of AGEs (advanced glycation end products) [7]. Increased concentrations of triose phosphate glycolytic intermediates (glyceraldehyde 3-phosphate and dihydroxyacetone phosphate) is the trigger for these processes [8,9]. A pharmacological strategy that countered triose phosphate accumulation in hyperglycaemia would suppress multiple pathogenic pathways and prevent the development of diabetic microvascular complications. Activation of the reductive pentose phosphate pathway by high-dose thiamine therapy may achieve this by increasing transketolase activity and stimulating the conversion of glyceraldehyde 3-phosphate and fructose 6-phosphate into ribose 5-phosphate (Scheme 1).

Key words: advanced glycation end product (AGE), diabetes, fructosyl-lysine, nephropathy, neuropathy, retinopathy, thiamine. 

Abbreviations used: AGE, advanced glycation end product; CEL, Nε-(1-carboxyethyl)lysine; CML, Nε-carboxymethyllysine; 3DG-H, 3-deoxyglucosone-derived hydromidazolone \[(\text{Nε-(C-hydro-5-(2,3,4-trihydroxybutyl)-4-imidazolon-2-yl)ornithine})\]; δDG-H, δ-deoxyglucosone derived hydromidazolone \[(\text{Nδ-(C-hydro-5-(2,3,4-trihydroxybutyl)-4-imidazolon-2-yl)ornithine})\]; MG-H1, methylglyoxal-derived hydromidazolone \[(\text{Nε-(C-hydro-5-(5-methyl-4-imidazolon-2-yl)ornithine})\]; STZ, streptozotocin.

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Table 1 | AGEs in proteins of renal glomeruli, retina, sciatic nerve and blood plasma of control and STZ-induced diabetic rats

- denotes that the concentration was below the limit of detection. Significance: *P < 0.05, **P < 0.01, ***P < 0.001 with respect to normal healthy controls. NS, not significant.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Study group</th>
<th>Renal glomeruli</th>
<th>Retina</th>
<th>Sciatic nerve</th>
<th>Plasma protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML (mmol/mol of Lys)</td>
<td>Control</td>
<td>0.269 ± 0.111</td>
<td>0.172 ± 0.051</td>
<td>0.151 ± 0.087</td>
<td>0.033 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.501 ± 0.186*</td>
<td>0.451 ± 0.291</td>
<td>0.437 ± 0.077**</td>
<td>0.062 ± 0.008***</td>
</tr>
<tr>
<td>CEL (mmol/mol of Lys)</td>
<td>Control</td>
<td>0.329 ± 0.102</td>
<td>0.339 ± 0.091</td>
<td>0.115 ± 0.069</td>
<td>0.008 ± 0.003</td>
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<tr>
<td></td>
<td>Diabetic</td>
<td>0.706 ± 0.047***</td>
<td>NS</td>
<td>0.519 ± 0.286**</td>
<td>0.017 ± 0.006***</td>
</tr>
<tr>
<td>G-H1 (mmol/mol of Arg)</td>
<td>Control</td>
<td>0.044 ± 0.029</td>
<td>0.552 ± 0.103</td>
<td>0.517 ± 0.238</td>
<td>0.275 ± 0.041</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.111 ± 0.067**</td>
<td>1.39 ± 0.89**</td>
<td>1.22 ± 0.55**</td>
<td>0.565 ± 0.206***</td>
</tr>
<tr>
<td>MG-H1 (mmol/mol of Arg)</td>
<td>Control</td>
<td>2.30 ± 0.25</td>
<td>1.88 ± 0.51</td>
<td>4.75 ± 2.74</td>
<td>1.45 ± 0.39</td>
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<tr>
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<td>Diabetic</td>
<td>3.23 ± 0.90</td>
<td>5.24 ± 2.34***</td>
<td>10.03 ± 0.66**</td>
<td>2.24 ± 0.38**</td>
</tr>
<tr>
<td>3DG-H (mmol/mol of Arg)</td>
<td>Control</td>
<td>0.186 ± 0.092</td>
<td>0.20 ± 0.09</td>
<td>2.85 ± 1.24</td>
<td>2.26 ± 0.89</td>
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<tr>
<td></td>
<td>Diabetic</td>
<td>0.172 ± 0.112</td>
<td>0.42 ± 0.15*</td>
<td>5.73 ± 0.72**</td>
<td>NS</td>
</tr>
<tr>
<td>FL (mmol/mol of Lys)</td>
<td>Control</td>
<td>0.974 ± 0.098***</td>
<td>2.59 ± 1.23**</td>
<td>3.69 ± 0.72***</td>
<td>7.35 ± 1.59***</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.233 ± 0.015</td>
<td>0.72 ± 0.21</td>
<td>0.49 ± 0.09</td>
<td>1.77 ± 0.36</td>
</tr>
</tbody>
</table>

Scheme 1 | Shunting of glycolytic intermediates from the Embden-Meyerhof pathway (dotted enclosure) to the reductive pentose phosphate pathway in anaerobic glycolysis

G-6-P, glucose 6-phosphate; F-6-P, fructose 6-phosphate; F-1,6-bis-P, fructose 1,6-bisphosphate; TPI, triose phosphate isomerase; DHAP, dihydroxyacetone phosphate; GA3P, glyceraldehyde 3-phosphate; PG, phosphoglycerate; PEP, phosphoenolpyruvate; R-5-P, ribose 5-phosphate.

Concentrations of AGES in renal glomeruli, retina, peripheral nerve and plasma proteins of STZ (streptozotocin)-diabetic rats and normal healthy controls

The effects of high-dose thiamine and Benfotiamine (7 and 70 mg/kg) therapy on the accumulation of AGES in renal glomeruli, retina, sciatic nerve and plasma proteins in the STZ-induced diabetic rat model with moderate insulin therapy were investigated after 24 weeks of diabetes (Table 1). Incipient nephropathy developed in the STZ-diabetic controls over a 24-week period, as judged by hyperfiltration and microalbuminuria, and both high-dose thiamine and Benfotiamine therapy prevented this [3].

The diabetic rats had the characteristics of the diabetic state: increased plasma glucose concentration and increased glycated haemoglobin HbA1. After 24 weeks of diabetes, HbA1 was 17.7 ± 1.6%, which was increased with respect to normal controls (9.0 ± 0.8%; P < 0.001). Most HbA1 reflects the presence of the fructosamine residues Nε-fructosyllysine and FL (Nε-fructosyl-lysine) [10]. FL residues were detected in protein extracts of rat tissues and plasma protein. The concentration of FL residues was highest in renal glomeruli (4.13 mmol/mol of Lys). The FL residue concentration was increased markedly in diabetic rats in renal glomeruli (6-fold), retina (3-fold), sciatic nerve (7-fold) and plasma protein (3-fold). There were, therefore, marked increases in early-glycation adduct concentrations in tissue and plasma proteins of diabetic rats, with respect to normal controls. This was suggested previously by immunoblotting detection of FL [11].

There were significant increases in diabetic rats in the levels of CML (Nε-carboxymethyl-lysine) residues in protein extracts of renal glomeruli (86%), retina (189%), sciatic nerve (216%) and plasma protein (64%). There were also significant increases in diabetic rats of CEL [Nε-(1-carboxyethyl)lysine] residues in protein extracts of renal glomeruli (115%), sciatic nerve (351%) and plasma protein (112%), but no significant increase in the retina. There were tissue-specific increases in hydroimidazolone residue concentrations for hydroimidazolones derived from methylglyoxal {MG-H1 \[Nε-(5-hydro-5-methyl-4-imidazolon-2-yl)ornithine]} and glyoxal \[G-H1 \[Nε-(5-hydro-4-imidazolon-2-yl)ornithine]} in diabetic rats with respect to normal...
controls. G-H1 was increased in the retina (152%), nerve (136%) and plasma protein (105%); MG-H1 was increased in renal glomeruli (195%), retina (279%), nerve (111%) and plasma protein (54%); and 3DG-H was increased in renal glomeruli (51%), retina (110%) and nerve (50%). High-dose therapy with both thiamine and the thiamine prodrug Benfotiamine at doses of 7 and 70 mg/kg respectively prevented the accumulation of AGEs in renal glomeruli, retina, sciatic nerve and plasma protein in a dose-dependent manner without reversing the increase in FL, as exemplified by the decreases in MG-H1 and CEL in renal glomeruli [3]. This accumulation of AGEs and FL residues may be linked to the development of retinopathy, nephropathy, neuropathy and generalized angiopathy. STZ-diabetic rats on insulin maintenance therapy for 24 weeks developed incipient nephropathy [12] (as was found in the present study), but they do not usually develop retinopathy or neuropathy in this period – more severe, untreated diabetes is required. AGE accumulation generally precedes the development of complications in this model of diabetes (including overt nephropathy), and AGEs may be causally linked to the development of complications rather than being indicators of complications status. The lack of accumulation of CML and CEL residues in the retina may be due to the high proteasomal proteolysis activity in the retina [13], such that only AGEs with the highest flux of formation increase significantly. Overall, the quantitative screening of glycation adducts in STZ-diabetic rats and controls has shown a marked increase in FL, hydroimidazolones, CML and CEL residues at the sites of development of microvascular complications and in blood plasma, consistent with a role for glycation in the development of vascular complications of diabetes.

The prevention of AGE accumulation in the glomeruli, retina, peripheral nerve and plasma protein by high-dose thiamine and Benfotiamine suggests that high-dose thiamine supplementation may prevent the development of diabetic complications. Indeed, evidence is now emerging that high-dose thiamine and Benfotiamine prevent the development of microvascular complications of diabetes [3,14,15]. The primary intervention by these agents in the STZ-diabetic rat model was the prevention of thiamine deficiency and induction of transketolase expression, with consequent activation of the reductive pentose phosphate pathway shunt [3]. It is remarkable that these effects were achieved by increasing the dietary availability of thiamine to diabetic rats by as little as 20 times the minimum daily allowance – although this was sufficient to prevent thiamine deficiency. Thiamine deficiency exacerbated the development of diabetic nephropathy. We therefore propose that clinically diabetic subjects should avoid becoming thiamine deficient, even weakly so, and that high-dose thiamine repletion should be considered for therapy to prevent the development of clinical microvascular complications of diabetes.

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References

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