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Benfotiamine Inhibits Intracellular Formation of Advanced Glycation End Products in vivo

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ABSTRACT

We have demonstrated previously that intracellular formation of the advanced glycation end product (AGE) N-epsilon-(carboxymethyl)-lysine (CML) inversely correlates with diabetic vascular complications independently from glycemia (Diabetologia 42, 603, 1999). Here, we studied the effect of benfotiamine, a lipid-soluble thiamine derivative with known AGE-inhibiting properties in-vitro on the intracellular formation of (CML) and methylglyoxal-derived AGE in red blood cells. Blood was collected from 6 Type 1 diabetic patients (2 m, 4 f, age 31.8 ± 5.5 years; diabetes duration 15.3 ± 7.0 years) before and after treatment with 600 mg/day benfotiamin for 28 days. In addition to HbA1c (HPLC), CML and methylglyoxal were measured using specific antibodies and a quantitative dot blot technique

While treatment with benfotiamin did not affect HbA1c levels (at entry: 7.18±0.86%; at conclusion 6.88±0.88%; p not significant), levels of CML decreased by 40 % (737 ± 51 arbitray unit/mg protein (AU) vs 470 ± 86 AU; p<0.001). The levels of intracellular methylglyoxal-derived AGE were reduced by almost 70 % (1628 ± 1136 AU vs 500 ± 343 AU; p < 0.01). The data indicate that thiamine derivatives are effective inhibitors of both intracellular glycoxidation and AGE formation.

INTRODUCTION

Intracellular formation of the advanced glycation end product (AGE) Ne-(carboxymethyl)lysine (CML) inversely correlates with diabetic vascular complications independently from glycemia (Figure 1) (1). Intracellular CML is generated by the oxidation of Amadori products or, alternatively, by lipid peroxidation (2,3). The dicarbonyl methylglyoxal is formed by non-oxidative fragmentation of glycolysis-derived triose phosphates (Figure 2) and is the most important intracellular AGE (4,5).

Thiamin is a potent AGE-inhibitor in-vitro (6), and benfotiamine, the lipid-soluble prodrug of thiamin, was shown to reduce CML and other AGE in target tissues of diabetic complications in-vivo (7). The possible mechanisms by which thiamin/benfotiamine are thought to reduce intracellular AGE formation, are shown in Figure 3. We studied the effect of benfotiamine, a lipid-soluble thiamine derivative with known AGE-inhibiting properties in-vitro on the intracellular formation of (CML) and methylglyoxal-derived AGE in red blood cells of patients with type 1 diabetes.

Methods

Study group: six patients (2 males, 4 females), age 31.8 ± 5.5 years; diabetes duration 15.3 ± 7.0 years. Treatment with 600 mg/day benfotiamine (Milgamma, Wörwag, Böblingen, Germany) for 28 days after informed consent and approval by the local ethics committee.

Venous EDTA-blood (3 ml) drawn before and at the end of the study, samples lysed and centrifuged, adjusted to identical hemoglobin concentrations. Quantitative immunoblotting carried out essentially as described before (1). Statistical analysis was performed using the alternate Welsh t test.

Conclusion

Thiamine derivatives, in particular the lipid.soluble prodrug benfotiamine, are effective inhibitor of intracellular formation of AGE and CML.

References

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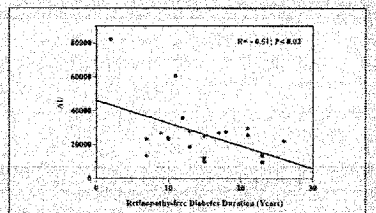


Fig. 1a: Correlation of retinopathy-free diabetes duration with the concentration of CML in memory T-cells. AU=arbitrary units (from Diabetologia 42, 603, 1999)

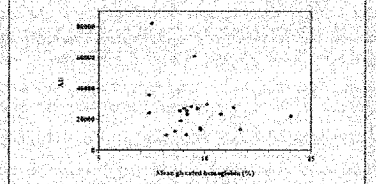


Fig. 1b: No correlation of mean glycated hemoglobin with CML levels in memory T-cells

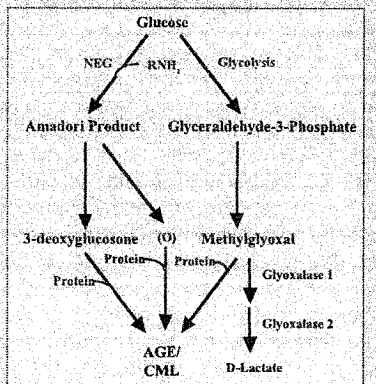


Fig. 2: Biochemical pathways of intracellular AGE/CML formation (Shinohara et al., JCI 101, 1142, 1998)

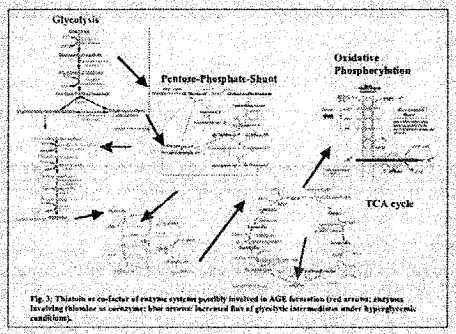
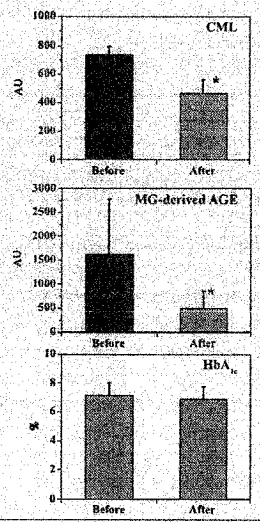


Fig. 3: Thiamin as cofactor of enzyme systems possibly involved in AGE formation (red arrows; enzymes involving thiamine as coenzyme; blue arrows: increased flux of glycolytic intermediates under hyperglycemic conditions).

Results of Benfotiamine treatment in Patients

Fig. 4



a. Significant reduction of CML in RBC after a 4-week treatment with benfotiamine. * p<0.001

b. Significant reduction of methylglyoxal-derived AGE in RBC after a 4-week treatment with benfotiamine. * p<0.01

c. No change in Amadori-product formation by benfotiamine treatment.