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SHORT COMMUNICATION

Oral benfotiamine plus α-lipoic acid normalises complication-causing pathways in type 1 diabetes

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Abstract
Aims/hypothesis We determined whether fixed doses of benfotiamine in combination with slow-release α-lipoic acid normalise markers of reactive oxygen species-induced pathways of complications in humans.
Methods Male participants with and without type 1 diabetes were studied in the General Clinical Research Centre of the Albert Einstein College of Medicine. Glycaemic status was assessed by measuring baseline values of three different indicators of hyperglycaemia. Intracellular AGE formation, hexosamine pathway activity and prostacyclin synthase activity were measured initially, and after 2 and 4 weeks of treatment.
Results In the nine participants with type 1 diabetes, treatment had no effect on any of the three indicators used to assess hyperglycaemia. However, treatment with benfotiamine plus α-lipoic acid completely normalised increased AGE formation, reduced increased monocyte hexosamine-modified proteins by 40% and normalised the 70% decrease in prostacyclin synthase activity from 1,709±586 pg/ml 6-keto-prostaglandin $F_{1\alpha}$ to 4,696±533 pg/ml.
Conclusions/interpretation These results show that the previously demonstrated beneficial effects of these agents on complication-causing pathways in rodent models of diabetic complications also occur in humans with type 1 diabetes.

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Keywords Advanced glycation endproducts · Benfotiamine · Diabetic complications · Hexosamine pathway · Hyperglycaemia · Lipoic acid · Prostacyclin synthase · Reactive oxygen species · Type 1 diabetes

Abbreviations
PGF prostaglandin F
PKC protein kinase C

Introduction

Benfotiamine blocks three major pathways of hyperglycaemic damage and prevents diabetic retinopathy and incipient nephropathy in experimental models [1, 2]. In cultured vascular cells, it also reduces aldose reductase gene expression and activity, as well as sorbitol levels [3]. It does so by activating the enzyme transketolase. α-Lipoic acid, a potent antioxidant, has also been reported to reduce diabetic microvascular and macrovascular complications in animal models [4, 5]. To determine whether benfotiamine in combination with α-lipoic acid would normalise markers of reactive oxygen species-induced pathways of complications in humans, we performed a pilot study in participants with type 1 diabetes using one daily dose of benfotiamine in combination with α-lipoic acid.

Methods

After protocol approval by the Committee on Clinical Investigations at the Albert Einstein College of Medicine, men with type 1 diabetes and matched healthy controls were recruited. Participant criteria included (in diabetic participants) diabetes duration between 0 and 15 years, current insulin therapy and no evidence of proliferative retinopathy, microalbuminuria, symptomatic diabetic neuropathy or cardiovascular disease. Participants taking any

medications and those with a history of smoking were excluded. Age of enrolled participants was 28.9±8.6 years, duration of diabetes was 25.5±7.9 years and BMI was 20.3±3.1 kg/m^2. All participants gave written informed consent.

The glycaemic status of study patients was assessed by measuring baseline values of HbA_{1c}, fructosamine and fasting plasma glucose. Mean HbA_{1c} was 8.7±0.7%, mean fructosamine was 421±29 μmol/l (normal range 174–286 μmol/l) and mean fasting blood glucose was 11±0.49 mmol/l.

At day 0, levels of markers of two benfotiamine-sensitive pathways were determined in participants: (1) intracellular AGE formation, as reflected by a marker of increased intracellular methylgloxal adducts in endothelial cells, angiopoietin 2 [6], and (2) hexosamine pathway activity, measured by determination of *N*-acetylglucosamine-modified protein in circulating monocytes [7]. Protein kinase C (PKC) activity in circulating monocytes could not be measured because the amount of blood required exceeded that approved by the Committee on Clinical Investigations. Serum levels of 6-keto-prostaglandin F $(PGF)_{1\alpha}$, a stable product produced by the non-enzymatic hydration of the anti-atherogenic mediator prostacyclin, were also determined [8]. Participants then took benfotiamine (300 mg twice a day; Advanced Orthomolecular Research, Calgary, AB, Canada), together with slow-release α-lipoic acid (600 mg twice a day; MRI, San Francisco, CA, USA) for 28 days. Blood was obtained at days 0, 15 and 28.

Data were analysed using one-factor analysis of variance to compare the means of all the groups. The Tukey–Kramer multiple comparisons procedure was used to determine which pairs of means were different.

Results

An initial study was performed with benfotiamine alone to determine whether the selected dose was sufficient to activate transketolase. In circulating monocytes, the dose of benfotiamine increased transketolase activity by two-fold to threefold within 2 weeks (1.45±0.17 vs 3.49±0.22 nmol min^{-1} [mg protein]$^{-1}$, mean±SEM), an effect similar to that observed in long-term diabetic rat models.

Following this, we examined the effect of combined treatment on angiopoietin-2, a marker of increased intracellular methylgloxal adducts in endothelial cells. Type 1 diabetes was associated with a 1.8-fold increase in circulating angiopoietin-2 levels (Fig. 1a). Treatment with benfotiamine plus α-lipoic acid completely normalised angiopoietin-2 levels by 2 weeks (2,416±312 vs 1,062±176 pg/ml, mean±SEM). Treatment had no effect on any of the three variables used to assess hyperglycaemia in this study (data not shown).

Next, we examined the effect of combined treatment on hexosamine pathway activity by measuring total N-acetylglucosamine-modified proteins in circulating monocytes (Fig. 1b). Type 1 diabetes was associated with a 2.8-fold increase in hexosamine pathway activity (3,838±765 vs 1,380±616 arbitrary units, mean±SEM). Two weeks of combined benfotiamine and lipoic acid treatment reduced this value by 40%.

Finally, type 1 diabetes was associated with a 70% decrease in activity of the critical endothelial anti-atherogenic

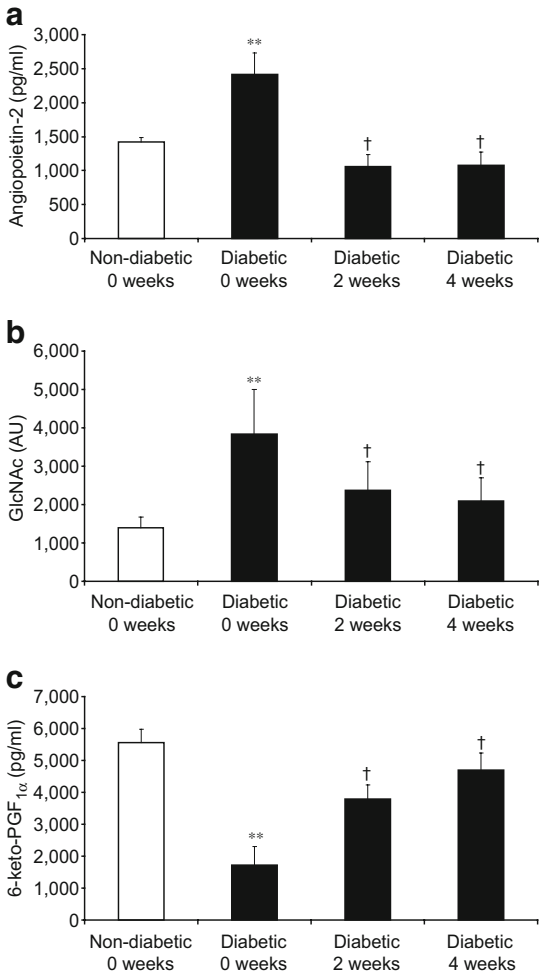

Fig. 1 **a** Angiopoietin-2 levels in serum of non-diabetic and type 1 diabetic participants before and during treatment with benfotiamine and α-lipoic acid. **b** Intracellular *N*-acetylglucosamine-modified protein (GlcNAc) in monocytes from non-diabetic and type 1 diabetic participants before and during treatment as above (**a**). **c** 6-keto-$PGF_{1\alpha}$ levels in serum of non-diabetic and type 1 diabetic participants before and during same treatment (**a**). Non-diabetic group n=12; type 1 diabetic group n=9; **p<0.01 compared with control; †p<0.01 compared with week 0. AU, arbitrary units

enzyme prostacyclin synthase from 5,775±294 pg/ml 6-keto-$PGF_{1\alpha}$, (mean±SEM) to 1,709±586 pg/ml 6-keto-$PGF_{1\alpha}$ (mean±SEM) (Fig. 1c). Treatment with benfotiamine plus α-lipoic acid normalised 6-keto-$PGF_{1\alpha}$ activity by 4 weeks (4,696±533 pg/ml 6-keto-$PGF_{1\alpha}$, mean±SEM).

Discussion

Increased hyperglycaemia-induced superoxide causes glycolytic intermediates to be shunted into the major pathways of hyperglycaemic damage [9]. These intermediates, which activate intracellular AGE formation, the hexosamine pathway and PKC, are also the final products of the transketolase reaction. Because of this, increasing transketolase activity via benfotiamine blocks these complications-causing pathways. Although the damaging pathways inhibited by benfotiamine have been a major focus of complications research, it is important to recognise that excess superoxide can damage vascular cells without involvement of any of these pathways. An important example is the oxidative inactivation of prostacyclin synthase, a critical anti-atherosclerosis endothelial enzyme [10]. For this reason, we combined the antioxidant α-lipoic acid with benfotiamine [4].

In this pilot study we report that treatment with oral benfotiamine plus α-lipoic acid normalises several complications-causing pathways in patients with type 1 diabetes. The incomplete normalisation of the hexosamine pathway in monocytes, which contrasts with its complete normalisation in rat retina [1], may reflect differential accumulation of benfotiamine in different cell types or a slow turnover of monocyte intracellular proteins. Together, these results show that the beneficial effects of these agents on these pathways, as seen in rodent models of diabetic complications [1], also occur in humans with type 1 diabetes. Replication of these results in a much larger study population after optimisation of benfotiamine and α-lipoic acid doses will be necessary in order to determine whether this treatment may help prevent diabetic retinopathy and nephropathy in human patients, as it does in diabetic animal models.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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