Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy

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Summary/Conclusion
Benfotiamine is a lipid soluble thiamine derivative, which gives much higher blood and tissue levels than do water-soluble thiamine derivatives. The results in both cultured endothelial cells and retinas from diabetic rats show that benfotiamine prevents activation of the hexosamine pathway, the intracellular AGE formation pathway and the DAG-PKC pathway. Benfotiamine blocks these pathways of hyperglycemic damage by increasing the activity of the pentose phosphate pathway enzyme transketolase. Benfotiamine also prevents hyperglycemia-induced activation of NF-κB in both cultured cells and diabetic retinas.

Administration of benfotiamine for nine month completetly prevented the development of experimental diabetic retinopathy in rats.

In summary benfotiamine is able to inhibit simultaneously three major pathways which are implicated in the pathogenesis of hyperglycemia induced vascular damage. This might be useful in preventing the development and progression of diabetic complications.

Introduction/Problem
Hyperglycemia have an important role in the pathogenesis of diabetic macrovascular disease. Four major metabolic mechanisms are there implicated: increased polyol pathway flux, increased hexosamine pathway flux, increased advanced glycation end product (AGE) formation and activation of protein kinase C (PKC) through diacylglycerol (DAG). In aortic endothelial cells the transcription factor NF-κB is also activated by hyperglycemia. Causally, hyperglycemia induce the overproduction of superoxide by the mitochondrial electron transport chain, which partially inhibits a glycolytic enzyme. Metabolites from glycolysis were therefore diverted into the four pathways that cause hyperglycemic damage.
Two of them are fructose-6-phosphate and glyceraldehyde-3-phosphate, which are produced by the thiamine-dependant enzyme transketolase. The transketolase reaction is determined by substrate concentration, which is increased in hyperglycemia. It is previously been reported that diabetic patients have subnormal transketolase activity.

Subject Matter/Aim of the Study
Benfotiamine, a lipid soluble thiamine derivative, has a greater bioavailability than does thiamine. In this study the effect of benfotiamine on transketolase activity in both cultured endothelial cells and animal retinal tissue is evaluated. The effect on hyperglycemia-induced activation of the hexosamine pathway, the intracellular AGE formation, the DAG-PKC pathway and the activation of NF-κB was also evaluated. Finally, the effect of benfotiamine administration on the development of experimental diabetic retinopathy was evaluated.

Study Design/Methodology
- Cell Cultures:
  For the benfotamine dose-response experiment, bovine aortic cells were incubated for 6 h with either 5 mM Glucose (glc), 30 mM glc or 30 mM glc + varying concentrations of benfotiamine (BT). Incubation for all other cell culture experiments: with either 5 mM glc, 30 mM glc, 5 mM glc + 25 mM mannitol, 30 mM glc + 50 µM BT, 30 mM glc + transketolase antisense + BT, 30 mM glc + transketolase antisense, 30 mM glc+ scrambled oligonucleotides or 30 mM glc + scrambled oligonucleotides + BT. Cells were incubated for 48 h prior to determination (det) of hexosamine pathway activity, for 5 d prior to det of AGE formation, for 3 d prior to det of PKC activity and 6 h prior to det of NK-κB activation.
- Transketolase acitivity was measured by adding 20 µl cytosolic fraction to 200 µl reaction mixture. The optical density was measured at 340 nm immediately and then every 10 min for 2 h. The activity was calculated from the difference in the optical density readings at 10 and 80 min using the extinction coefficient for NAD.

- Hexosamine pathway activity was assessed by UDP-N-acetylglucosamine (UDP-Glc-Nac) concentrations. After cell homogenization and centrifugation UDP-Glc-Nac in the supernatant was determined by HPLC.
- Advanced glycation end products: cell extract protein was used for quantitative immunoblotting. Immunocomplexes were visualized and quantified using the enzyme-catalyzed flourescence, as well as retinal extracts.
- Protein kinase C-activity was assessed using the Protein Kinase C Assay System.
- NF-κB activation: a flourescence DNA-protein binding assay was performed in cultured cells. An electrophoretic mobility shift assay was used in the retina.
- Animals: Diabetes was induced by i.v. injection of Streptozotocin in 6-week-old male Wistar rats. Hyperglycemic (blood glc > 15 mM, 15 d after injection) rats were randomly assigned to receive either standard rat chow containing benfotiamine or no treatment. Control: non-diabetic animals. Body weight and blood glucose were measured at regular intervals. Glycosylated hemoglobin was measured at the end of the study using affinity chromotography.
- Retinal preparations: after 36 weeks of hyperglycemia, eyes were enucleated under deep anesthesia. Retinas from the left eye were dissected, snap frozen and stored until analysis for transketolase activity, hexosamine pathway activity, AGE formation, PKC activity and NF-κB activation. Retinas of the right eyes were subjected to an established enzymatic digestion method.

Study Results

- Activation of transketolase in endothelial cells:
  Benfotiamine concentrations of both 50 µM and 100 µM increased transketolase activity in cells by four-fold. 50 µM benfotiamine is therefore used in cell culture experiments.
- Prevention of hyperglycemic damage in vitro: Hexosamine pathway (indicator UDP-Glc-NAc): Incubation of bovine aortic endothelial cells with 30 mM Glc increased UDP-Glc-NAc concentration five-fold (compared to 5 mM glc). This increase was completely prevented by 50 µM benfotiamine.
- AGE formation:
  Incubation with 30 mM glc increased intracellular AGE-formation 2,5 fold (compared to 5 mM glc). The increase was completely prevented by 50 µM benfotiamine. Membrane PKC-activity: incubation with 30 mM glc increased the membrane fraction of intracellular PKC activity 2.1-fold (compared to 5 mM glc). The increase was completely prevented by 50 µM benfotiamine.
- Effect of benfotiamine on hyperglycemia-induced activation of NK-κB activating transketolase: incubation with 30 mM glc increased Nk-κB activation by 2.1 fold. Benfotiamine completely prevented this effect.
- The inhibitory effect of benfotiamine on all these hyperglycemia-induced changes was completely blocked in the presence of antisense-transketolase. It showed that benfotiamine blocks these pathways by activating transketolase.
- Activation of transketolase in diabetic retinas: After 36 weeks of hyperglycemia, retinal transketolase activity was reduced. Benfotiamine treatment of diabetic rats for 36 weeks increased transketolase activity in the retina by 2.5-fold compared with untreated diabetics.
- Prevention of pathway activation in diabetic retinas: Diabetes increased hexosamine pathway activity in retina tissue three-fold, increased AGE-formation and membrane PKC activity and increased NF-κB activation in the retina three-fold compared with retinas from non-diabetic animals. Benfotiamine treatment reduced the UDP-GlcNAc levels below those observed in non-diabetic rats. It normalized AGE levels and and PKC activity. Benfotiamine treatment prevented the diabetes-induced activation of NF-κB in the retinals of long-term diabetic rats.
- Prevention of experimental diabetic retinopathy: quantitative morphological studies on retinas after 36 weeks of diabetes showed that the number of acellular capillary segments in retinal vessels increased to over 3 times than found in non-diabetics. With 36 weeks of diabetes and with benfotiamine treatment the number of retinal acellular capillary segments did not change from that found in non-diabetics.