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ORIGINAL ARTICLE

Benfotiamine reduces genomic damage in peripheral lymphocytes of hemodialysis patients

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Abstract Hemodialysis patients have an elevated genomic damage in peripheral blood lymphocytes (PBLs) and an increased cancer incidence, possibly due to accumulation of uremic toxins like advanced glycation end products (AGEs). Because the vitamin B1 prodrug benfotiamine reduces AGE levels in experimental diabetes, and dialysis patients often suffer from vitamin B1 deficiency, we conducted two consecutive studies supplementing hemodialysis patients with benfotiamine. In both studies, genomic damage was measured as micronucleus frequency of PBLs before and at three time-points after initiation of benfotiamine supplementation. AGE-associated fluorescence in plasma, and in the second study additionally, the antioxidative capacity of plasma was analyzed. Benfotiamine significantly lowered the genomic damage of PBLs in hemodialysis patients of both studies independent of changes in plasma AGE levels. The second study gave a hint to the mechanism, as the antioxidative capacity of the plasma of the treated patients clearly increased, which might ameliorate the DNA damage.

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Introduction

End-stage renal disease (ESRD) is characterized by a high incidence of cancer (Maisonneuve et al. 1999, Stewart et al. 2003, Teschner et al. 2002). Especially, the risk to develop tumors of the kidney, bladder, and oral cavity is up to nine times higher than it is for the general population (Teschner et al. 2002). An increase of genomic damage in peripheral blood lymphocytes (PBLs) of ESRD patients was documented by single-cell gel electrophoresis (Kan et al. 2002, Stopper et al. 2001) and by the micronuclei (MN) frequency test (Fragedaki et al. 2005, Stopper et al. 1999). The observation of increased DNA damage in ESRD is further supported by the determination of the oxidative DNA adduct 8-hydroxy 2′-deoxyguanosine (8-OHdG) in leukocytes (Tarng et al. 2000), the analysis of sister chromatid exchange and chromosomal aberrations (Buemi et al. 2006, Cengiz et al. 1988), as well as the demonstration of mitochondrial DNA deletions (Lim et al. 2002, Liu et al. 2001). An important clinical consequence of genomic damage is the later development of cancer. Recently, prospective epidemiological studies showed a correlation between medium and high MN frequencies and a significant increase of all cancers (Bonassi et al. 2007).

The enhanced DNA damage in ESRD is most probably caused by several mechanisms. These patients suffer from increased oxygen radical formation in the presence of a reduced antioxidant defense (Morena et al. 2002, 2005), chronic microinflammation, and infections (Vamvakas et al. 1998), from an impaired DNA repair

(Zevin et al. 1991), as well as from accumulation of uremic toxins (Boure and Vanholder 2004). Uremic toxins are compounds, retained in the course of ESRD, which disturb numerous biological functions. Up to now, 90 organic compounds in uremia have been identified (Vanholder et al. 2003). Among them, a variety of advanced glycation end products (AGEs) are listed, belonging to the middle molecule toxicity category of uremia (Schinzel et al. 2001).

AGEs are formed by non-enzymatic reactions between reducing sugars and free amino groups of proteins (Schleicher et al. 2001). The formation and accumulation of AGEs progresses at an accelerated rate in uremia (Glorieux et al. 2004). AGEs are implicated in the pathogenesis of the uremic syndrome, e.g., dialysis-related amyloidosis, dyslipidemia, and vascular dysfunction (Schwenger et al. 2001). Also, diabetic patients suffer from an accelerated accumulation of AGEs, which are involved in diabetic complications like atherosclerosis and diabetic microangiopathy (Bierhaus et al. 1998, Yamagishi et al. 2003). If these patients develop renal failure, an additional rise of AGEs occurs due to insufficient removal (Agalou et al. 2005).

In the pathogenesis of vascular and renal complications (Raj et al. 2000), AGE-induced oxidative stress in cells via the interaction with the receptor RAGE (receptor of advanced glycation end products), is of fundamental importance (Yamagishi et al. 2003). This amplifies the oxidative stress already increased in the diabetic milieu and uremia (Ceriello et al. 2001, Himmelfarb et al. 2002, Morena et al. 2005). The generated free radicals could also be responsible for the increased genomic damage of these patients (Croteau and Bohr 1997). In cultivated mammalian cells, a genotoxicity could be shown for the specific AGEs carboxymethyllysine and methylglyoxal, as well as for a mixture of unspecified AGEs (Stopper et al. 2003). This AGE-induced genotoxicity could be reduced by the addition of the antioxidant *N*-acetylcysteine, implying an important role of oxidative stress.

It has been demonstrated in diabetic rats and in cell culture that the lipophilic vitamin B1 (thiamine) prodrug benfotiamine, in high doses, reduced AGE levels and microangiopathy (Hammes et al. 2003, Pomero et al. 2001). In addition, clinical studies in diabetics imply an amelioration of diabetic neuropathy (Haupt et al. 2005, Winkler et al. 1999), as well as an improvement of thiamine deficiency in ESRD patients (Frank et al. 2000). Based on these observations, the effect of benfotiamine, in a high dose, on genomic damage and AGE plasma levels of diabetic and nondiabetic hemodialysis patients was analyzed in two prospective studies: one pilot prospective study with 15 patients followed by a single-blind placebo-controlled prospective study with 23 participants.

Subjects and methods

Subjects

Two prospective intervention studies were carried out to observe the effect of benfotiamine on the DNA damage of PBLs of hemodialysis patients. The first was a pilot study with 15 patients, the second a single-blind, placebo-controlled study with 23 patients (15 treated with benfotiamine and 8 with placebo). All participants were stable long-term patients of the hemodialysis program of the KfH-Nierenzentrum Würzburg e.V. (Kuratorium für Dialyse und Nierentransplantation). Exclusion criteria were bacterial or viral infections, and malignancies in the last 10 years. All patients were treated with synthetic, biocompatible high-flux dialyzers, with polysulfone membranes (FX 60, Fresenius Medical Care, Bad Homburg, Germany).

Both studies were approved by the Ethics Committee of the University of Würzburg and were conducted in accordance with the current version of the Declaration of Helsinki. The participants gave their written informed consent before participating in the studies.

Pilot prospective study

Fifteen patients were included in the first study. The patients' demographic data, time on hemodialysis, and primary kidney disease are summarized in Table 1. The average age at the beginning of the study was 63.0±4.2 years, and the average time on hemodialysis for the six female and nine male patients was 5.7±1.3 years. Five patients suffered from diabetes mellitus.

Table 1 Characteristics of the pilot prospective study group of hemodialysis patients and of the verum group and the placebo group of the single-blind placebo-controlled prospective study in hemodialysis patients

Characteristics	Pilot prospective study group	Single-blind placebo-controlled prospective study group	
		Verum	Placebo
Number	15	15	8
Age (years)	63.6±4.2	66.1±2.6	64.9±3.8
Gender (male/female)	9/6	9/6	4/4
Diabetes mellitus (yes/no)	5/10	6/9	4/4
Time on hemodialysis (years)	5.7±1.3	5.5±1.1	6.6±2.2
Causes of renal failure			
Glomerulonephritis	6	5	4
Diabetic nephropathy	3	4	2
Other	6	6	2

The patients received benfotiamine as milgamma® mono 150 provided by Wörwag Pharma GmbH & Co. KG (Böblingen, Germany). The starting dose was 300 mg daily for the first 6 weeks, followed by a higher dose of 450 mg until the end of the study.

Single-blind placebo-controlled prospective study

Twenty-three patients were included in the second study. After the first analysis of their micronucleus frequency, they were matched for micronucleus frequency, age, and time on dialysis. Based on this, they were divided into two groups: one with 15 patients receiving the dose of 600 mg benfotiamine per day and the second group with 8 patients receiving placebo. The patients' demographic data, time on hemodialysis, and primary kidney disease are summarized in Table 1. The average age at the beginning of the study was 66.1±2.6 years in the treatment group and 64.9±3.8 in the placebo group. The treatment group was comprised of nine male and six female patients, and the placebo group of four male and four female patients. The average time on hemodialysis was 5.5±1.1 years in the treatment group and 6.6±2.2 in the placebo group. In the treatment group, 6 of the 15 patients suffered from diabetes mellitus and in the placebo group 4 of the 8 patients. The body mass index (BMI) of the treatment group was slightly higher than the one from the placebo group (27.0±1.3 and 24.2±2.1, respectively).

Material

If not otherwise mentioned, chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Collection of blood samples

Blood samples (9 ml) were taken before the dialysis session via an indwelling cannula and collected in heparin-containing tubes. The samples were transported at room temperature to the Department of Toxicology for immediate isolation of PBLs. To determine the basal micronucleus frequencies, three blood samples of each patient were taken in 1-week intervals. After initiation of vitamin supplementation, three further samples in approximately 6-week intervals were taken. Routine chemistry tests and the assay of ferric reduction activity of plasma were performed on the day of blood collection. For the AGE analysis, aliquots of plasma were stored at −20°C.

Cell isolation

PBLs were isolated by standard density gradient centrifugation using FicoLite H (Linaris-H, Wertheim, Germany). In brief, the blood was layered (1:1) onto FicoLite and centrifuged at 1,600 rpm (370×*g*) for 30 min at room temperature. The PBL layer was removed, and the cells were washed twice (1,300 rpm; 250×*g*; 10 min) with RPMI-1640.

Culture conditions

PBLs were cultured at a cell density of 10^6 cells/ml in 5 ml of culture medium at 37°C in a humified, 5% CO_2 incubator. The culture medium consisted of RPMI-1640, supplemented with 15% fetal bovine serum, L-glutamine, Na-pyruvate, antibiotics (penicillin/streptomycin/tylosine), and nonessential amino acids. For mitotic activation of lymphocytes, phytohemagglutinine (PHA, final concentration 10 µg/ml) was added.

Micronucleus frequency test

Micronuclei are expressed in dividing cells and either contain chromosome breaks or whole chromosomes that are unable to travel to the spindle poles during mitosis (Stopper and Müller 1997). They are observed in cells with completed nuclear division and are counted in a binucleated stage of the cell cycle after using the cytokinesis inhibitor, cytochalasin B. The micronuclei frequency assay was performed according to Fenech et al. (2003) with slight modifications. For this, cytochalasin B (final concentration 5 µg/ml) was added to the cultures at 44 h after stimulation with PHA. At 72 h after culture initiation, the cells were spun on glass slides with a cytocentrifuge (Shandon, Cytospin 3; 1,000 rpm; 145×*g*; 5 min). Subsequently, fixation in cold (−20°C) methanol was performed for at least 2 h. Slides were stored at −20°C in sealed boxes. Before evaluation, slides were stained with acridineorange (0.00625% in Sørensen buffer, *w*/*v*, ph 6.8) for 5 min and washed twice in Sørensen buffer.

From each blood sample, 1,000 binucleated lymphocytes were screened for the presence of micronuclei with fluorescence microscopy at ×400 magnification. The scoring criteria corresponded to those described by Fenech et al. (2003). Objects were classified as micronuclei if they appeared separated from the nucleus, were round or oval, showed staining characteristics similar to those of the nuclei, and had an area less than one fourth of the area of the average normal nucleus.

AGE-associated fluorescence

AGE-associated fluorescence was measured by fluorescence spectroscopy (λ_{ex} 350 nm/λ_{em} 450 nm) of 50-fold diluted plasma samples in triplicate, as described previously (Munch et al. 1997).

Ferric reducing ability of plasma assay

The antioxidative capacity of plasma was determined as described by Benzie and Strain (1996).

Blood chemistry parameters

Transketolase activity, thiamine pyrophosphate (TPP) effect, and vitamin B1 content were determined by a specialized laboratory (Medizinisches Labor Bremen, Bremen, Germany). HbA_{1c}, creatinine, total protein, albumin, and C-reactive protein (CRP) were determined according to standard procedures in the Institute of Clinical Biochemistry and Pathobiochemistry of the University of Würzburg.

Statistics

Data are presented as means±SE. The means were compared for significance between groups using the two-sided Student's *t* test and in-between one group with the paired Student's *t* test. *p* values≤0.05 were considered significant.

Results

The effect of a supplementation with a lipid-soluble form of vitamin B1 (thiamine), benfotiamine, on genomic damage in PBLs of dialysis patients was first analyzed in a prospective pilot study with 15 participants. The characteristics of the group are given in Table 1.

For the determination of a basal value of genomic damage, the mean micronucleus frequency of lymphocytes, taken at three time-points before the supplementation with benfotiamine, was determined. After distribution of the vitamin to the participants and a break of 6 weeks, three blood samples at intervals of 4–6 weeks were analyzed. As can be seen in Table 2, the intake of benfotiamine significantly decreased the micronucleus frequency of all patients compared to the basal value. This significance persisted after division of the group into diabetics and nondiabetics (Fig. 1A), showing a beneficial effect of benfotiamine not only for patients suffering from diabetes mellitus.

Because benfotiamine is known to have effects on AGE levels which are increased in diabetes and uremia, the plasma level of AGE-associated fluorescence was measured in the study group. Table 2 shows that benfotiamine in this study had no impact on the plasma AGE concentration. This hints to another mechanism being responsible for its genome protective effect.

Benfotiamine had no impact on the microinflammatory state of the participants (Table 2). However, the concentration of total plasma protein rose in the whole group, with

Table 2 Micronucleus frequency (micronuclei/1,000 binucleated cells —MN/1,000 BN), advanced glycation endproduct (AGE)-associated fluorescence (arbitrary units—AU), C-reactive protein (CRP) and plasma total protein concentration before and after supplementation of the patients of the pilot prospective study with benfotiamine

Pilot prospective study group	Before	After
Micronucleus frequency (MN/1,000 BN)	16.0 ± 1.2	11.1 ± 1.1*
AGE content (AU)	1,179 ± 65	1,187 ± 56
CRP (mg/l plasma)	6.7 ± 1.7	5.6 ± 1.3
Total protein (g/l plasma)	58.6 ± 1.5	60.5 ± 2.6
Total protein (g/l plasma) diabetic patients	60.7 ± 2.9	65.7 ± 3.4; $p = 0.07$

*$p < 0.001$ vs. basal value

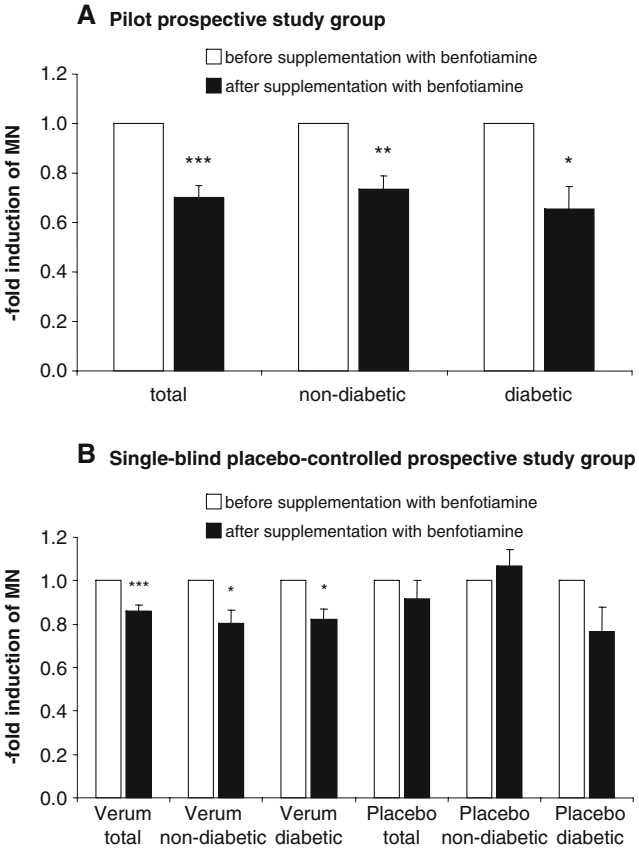

Fig. 1 **a** Micronucleus frequencies before and after supplementation with benfotiamine of the total pilot prosective study group, and subdivided in nondiabetic and diabetic subjects. Shown is the -fold induction of micronuclei (*MN*), related to the basal *MN* frequency before supplementation with benfotiamine. **b** Micronucleus frequencies before and after supplementation with benfotiamine of the total verum and placebo groups of the single-blind placebo-controlled prospective study, and of the two groups subdivided in nondiabetic and diabetic subjects. Shown is the -fold induction of micronuclei (*MN*), related to the basal *MN* frequency before supplementation with benfotiamine. *$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. basal MN value

a near-significant increase (P=0.07) in the diabetic subgroup.

We could show in in vitro studies that benfotiamine has a direct antioxidative capacity (Schmid et al. 2008). Antioxidants are able to decrease micronucleus frequencies (Volkovova et al. 2005). To confirm the results of the pilot study and to further elucidate the mechanism by which benfotiamine decreases micronucleus frequencies, a second study with a different design was conducted: a single-blind placebo-controlled prospective study with 23 patients. Again micronucleus frequencies and AGE-associated fluorescence were analyzed. In addition, the antioxidative capacity of the plasma was measured and the patients' compliance to benfotiamine was assessed by quantifying plasma vitamin B1 levels. Furthermore, the improvement of a possible thiamine deficiency was determined by measurement of transketolase activity.

No significant deviation in mean age and time on dialysis existed between the treatment and the placebo groups (Table 1). There was a slight imbalance between the proportions of diabetics and women in the groups, which had no impact on any of the measured end points.

As can be seen in Table 3, the compliance of the study participants to benfotiamine was good. The plasma vitamin B1 content of the treatment group rose significantly: 18-fold compared to the levels before benfotiamine supplementation. The treated group reached 45-fold higher plasma concentrations than the placebo group, where the patients suffered from a significant loss of plasma vitamin B1. Also, the parameters for the evaluation of a thiamine deficiency, transketolase activity and thiamine pyrophosphate (TPP) effect were improved in the treatment group, while those of the placebo group declined.

As was the case in the pilot study, the basal micronucleus frequency was determined at three time-points before the supplementation with benfotiamine. The micronucleus frequency from the first time-point was used to split the participants into two groups, a treatment group receiving benfotiamine and a placebo group, matched for mean micronucleus frequency, age, and time on dialysis. Again, three blood samples at intervals of 4–6 weeks were analyzed, starting 6 weeks after the distribution of benfotiamine and placebo to the participants. The micronucleus frequency was reduced significantly in patients taking benfotiamine compared to their starting value, while the micronucleus frequency of the placebo group showed no significant changes (Table 3). When subdivided into patients with and without diabetes and compared to the basal values, the micronucleus frequency was significantly reduced in both subgroups of the treatment group (Fig. 1B). A subdivision of the placebo group revealed a different behavior of the micronucleus frequencies in subjects with diabetes, where the number of micronuclei was decreased without reaching statistical significance, compared to subjects without diabetes, where the micronuclei number was increased.

Table 3 Plasma vitamin B1 concentration, transketolase activity and thiamine pyrophosphate effect (TPP effect), micronucleus frequency (micronuclei/1,000 binucleated cells—MN/1,000 BN), advanced glycation end product (AGE)-associated fluorescence (arbitrary units—AU), and ferric-reducing ability of plasma (FRAP), as well as C-reactive protein (CRP) and plasma total protein concentration of the verum and the placebo groups of the single-blind placebo-controlled prospective study before and after supplementation with benfotiamine

Single-blind placebo-controlled prospective study group		Before	After
Vit B1 content (μg/l plasma)	Verum	124±60	2,278±519*·****
	Placebo	72.6±7.7	50.0±5.8*
Transketolase activity (U/l)	Verum	73.7±4.8	76.9±3.9
	Placebo	81.0±4.4	77.0±5.3
TPP effect (%)	Verum	3.7±1.1	3.0±0.9
	Placebo	2.6±0.7	6.9±3.0
Micronucleus frequency (MN/1,000 BN)	Verum	22.5±1.5	19.2±1.5**
	Placebo	22.5±2.8	20.8±3.2
AGE concentration (AU)	Verum	3,061±159	2,239±128***
	Placebo	3,469±193	2,475±220**
HbA_{1c} (%)	Verum	5.7±0.3	6.1±0.4
	Placebo	5.8±0.5	5.6±0.4
FRAP (nmol/l plasma)	Verum	1,218±41	1,291±37; p=0.058
	Placebo	1,425±119	1,485±108
CRP (mg/l plasma)	Verum	15.1±4.3	10.9±2.7
	Placebo	16.3±6.7	26.3±7.7
Total protein (g/l plasma)	Verum	67.9±1.3	69.3±1.4*
	Placebo	70.4±2.1	70.6±2.2

*≤0.05
**p<0.01
***p<0.001 vs. basal value
****p≤0.05 vs. placebo group

The analysis of AGE-associated fluorescence in the plasma of the patients showed a significant decline of AGEs in the treatment group and in the placebo group (Table 3). HbA_{1c}, a determinant of the quality of diabetes suppression and itself a glycated protein, did not change significantly during the time of the study. The antioxidative capacity of the plasma, measured as ferric-reducing ability of plasma (FRAP), was raised in both groups, but more pronounced in the treatment group, where the increase almost reached significance (P=0.058).

In this study, the amount of total protein was once again higher in the treatment group at the end of the supplementation with benfotiamine, compared to the amount at the beginning of the study (Table 3). This time round, the difference was not as high, but because of a lower variation, it was statistically significant for the whole group. Although the indicator of inflammation, CRP was decreased in the

treated group and increased in the placebo group; this was not a significant effect and showed no correlation to the micronucleus frequency.

A significant correlation was found between the micronucleus frequency and transketolase activity (Fig. 2).

Discussion

Two studies were conducted to investigate, for the first time, the effect of the lipophilic vitamin B1 prodrug benfotiamine on the genomic damage of PBLs of dialysis patients with and without diabetes mellitus. In both studies, the supplementation with benfotiamine led to a decrease of micronucleus frequencies compared to the basal values of both studies. To strengthen the effect observed in the pilot study and to affirm the differences measured in the micronucleus frequency, the second study was designed as a single-blind, placebo-controlled investigation. The absence of significant changes of the micronucleus frequency in the placebo group confirm the decrease of the micronuclei number to be a consequence of the benfotiamine supplementation. In all, the changes are small in the pilot study, which is most probably caused by the already low micronucleus frequencies of the participants, but nevertheless statistically significant.

Micronuclei originate from chromosome fragments or whole chromosomes not included in the daughter nuclei during cell division. This event can be induced by oxidative stress, exposure to genotoxic substances, genetic defects in cell cycle genes or DNA repair genes, and nutritional deficiencies affecting DNA metabolism and chromosome segregation (Fenech et al. 2005). The assessment of the micronucleus frequency in PBLs is extensively used to evaluate the presence and extent of chromosomal damage in populations exposed to genotoxic agents or bearing a susceptible genetic profile (Fenech et al. 1999). Recently, a follow-up of 6,718 subjects from middle Europe supported the hypothesis that the micronucleus frequency in PBLs is a predictive biomarker of cancer risk (Bonassi et al. 2007). Because the micronucleus frequency is also sensitive to small variations in micronutrient status within the physiological range, it is an excellent biomarker for identifying dietary factors essential for genome stability (Fenech 2002). It therefore may be an early biomarker for the investigation of protective effects of therapeutic alterations in dialysis patients, who show an increased cancer incidence (Stewart et al. 2003).

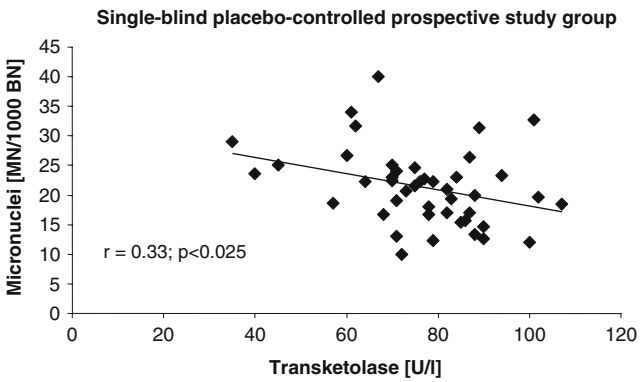

Fig. 2 Micronucleus frequency in relation to transketolase activity. Shown are means of micronuclei (*MN*) per 1,000 binucleated lymphocytes (*BN*) for each patient of the single-blind placebo-controlled prospective study from the three sampling time-points before and after supplementation with benfotiamine correlated with the transketolase activity before and after supplementation with benfotiamine

Benfotiamine might have an influence on two of the multiple mechanisms which probably lead to the elevated cancer risk of ESRD patients: on the formation of AGEs and on the generation of oxidative stress.

Benfotiamine upregulates the activity and also the expression of transketolase, by providing the enzyme's cofactor thiamine pyrophosphate (Babaei-Jadidi et al. 2003). Transketolase is the rate-limiting enzyme which deflects glyceraldehyde 3-phosphate and fructose 6-phosphate away from glycolysis into the reductive branch of the pentose phosphate pathway (Gadau et al. 2006). This prevents inter alia the accumulation of triosephosphates, which is a key feature of biochemical dysfunction in hyperglycemia and leads to increased formation of AGEs (Babaei-Jadidi et al. 2003). Several studies showed that transketolase activity in ESRD patients deviates from healthy subjects (Frank et al. 2000, Pietrzak and Baczyk 1997). Although thiamine deficiency is mainly encountered in alcoholics, it may complicate chronic dialysis because of low intake and accelerated loss of thiamine during dialysis, and can lead to encephalopathies whose cause is often not recognized (Hung et al. 2001a, b). Of the dialysis patients in the placebo-controlled study, only three displayed a transketolase activity beneath the reference value. Nevertheless, almost all treated subjects showed an increase of transketolase activity, not observed in the placebo group. The markedly increased plasma vitamin B1 levels of the treated patients together with the rise in transketolase activity can be seen as proof of patient compliance. According to a recent study, the real plasma concentration of the patients cannot be estimated by measuring erythrocyte transketolase activity, the conventional assessment of thiamine status. Due to an active transport of thiamine out of the plasma, the erythrocytes of patients maintain a normal concentration of thiamine while the plasma concentration of thiamine was severely decreased (Thornalley et al. 2007). So, it could be that the patients in this study also had lower thiamine levels than were measured routinely.

Several authors report a reduction of AGE levels either in animals or in cell culture, related to the administration of thiamine or benfotiamine, which they ascribe to the induction of transketolase activity (Hammes et al. 2003, Pomero et al. 2001). We could not see a significant decrease of AGEs in the patients' plasma, differing from placebo. This might be due to the fact that we only quantified the AGE-associated fluorescence, produced by a number of nonspecified glycated products. Other studies either measured specific AGEs, such as methylglyoxal or carboxyethyllysine by mass spectrometry (Babaei-Jadidi et al. 2003), or used antibodies raised against AGEs mainly consisting of methylglyoxal or carboxymethyllysine (Gadau et al. 2006, Hammes et al. 2003). It might be that only these specific AGEs are lowered by benfotiamine or that the reduction of these specific AGEs is not seen in the total AGE-associated fluorescence. On the other hand, there are two reports of studies conducted in mice, with no observable reduction of AGEs by benfotiamine treatment, independent of the amelioration of diabetic neuropathy and cardiomyopathy (Ceylan-Isik et al. 2006, Wu and Ren 2006). In addition, it has to be noted that until now no author has published evidence of a decrease of AGEs under therapy with either thiamine or benfotiamine in humans. Only one study reports an acute prevention of the increase of methylglyoxal and carboxymethyllysine in serum after the ingestion of a meal rich in AGEs (Stirban et al. 2006), while in another study, no decrease of the AGE pentosidine was seen after 8 weeks of 250 mg thiamine/day (Nascimento et al. 2006). Based on these reports, potential effects of thiamine or benfotiamine on AGE levels in humans still require confirmation. As yet, the parallel significant decrease of AGE-associated fluorescence in both the treatment and placebo groups cannot be explained. Theoretically, there might be seasonal influences on the plasma AGE level. However, published evidence is lacking. One study commenting on this found no seasonal variations (Stamatas et al. 2006). However, the authors measured the AGE load as skin fluorescence, and there is no experience yet how fast this marker reacts to changes of the plasma AGE concentration. Another explanation might be a higher dietary discipline of all individuals of the study, due to the participation in our study.

Because we observed a significant negative correlation between micronucleus frequency and transketolase activity without a decrease of AGEs, another effect of the benfotiamine-mediated induction of transketolase might play a role in the lowering of genomic damage. The reductive pentose phosphate pathway, which is accelerated by an activated transketolase, is a major producer of reduced NAD(P)H, thereby contributing to the antioxidative defense of the cells (Siems et al. 2000). The antioxidative capacity of the participants' plasma was almost significantly higher after the supplementation with benfotiamine than before. This might have been connected to the upregulation of transketolase, but could have also been a positive side effect of benfotiamine, which we could show to have an intrinsic antioxidative activity itself (Schmid et al. 2008).

Another possible positive side effect of benfotiamine, especially for dialysis patients, might be the slight increase of total protein, which we observed in both studies. The plasma protein concentration (in particular albumin) is an important marker of the nutritional state and an indicator of microinflammation (Basile 2003, Thijssen et al. 2007). For this reason, the rise of total protein concentration is desirable. In diabetic rats, a decline of urinary protein excretion was detected, indicating a beneficial effect of benfotiamine on renal function (Babaei-Jadidi et al. 2003). Because the participants of our studies did not have residual renal function, another mechanism for the prevention of protein loss must exist which leads to higher plasma protein concentration, such as an improvement of the negative acute phase response or a better nutritional state.

In summary, our data suggest that a supplementation with benfotiamine in a high dose for approximately 4 months lowers the enhanced genomic damage in PBLs of hemodialysis patients. The reduction was associated with a rise of transketolase activity and an improvement of antioxidative defense. Whether the enhanced cancer incidence of ESRD patients is reduced in the long-run has to be elucidated in long-term investigations.

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