Preventive effects of benfotiamine in chronic diabetic complications

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ABSTRACT

Aims/Introduction: In diabetes, increased oxidative stress as a result of damage to the electron transport chain can lead to tissue injury through upregulation of multiple vasoactive factors and extracellular matrix proteins. Benfotiamine, a lipid soluble thiamine derivative, through reducing mitochondrial superoxide production, blocks multiple pathways leading to tissue damage in hyperglycemia. We investigated if treatment with benfotiamine can prevent diabetes-induced production of vasoactive factors and extracellular matrix proteins, and whether such effects are tissue-specific. We also examined whether effects of benfotiamine are mediated through a nuclear mechanism.

Materials and Methods: Retinal, renal and cardiac tissues from the streptozotocin-induced diabetic rats were examined after 4 months of follow up. mRNA levels were quantified using real-time RT-PCR. Protein levels were quantified using western blot and ELISA. Cellular expressions of 8-Hydroxy-2′-deoxyguanosine, a marker of nuclear DNA damage and Phospho-H2AX were also examined.

Results: Diabetic animals showed hyperglycemia, glucosuria, increased urinary albumin/creatine ratio and loss of bodyweight. In the kidneys, heart and retina, diabetes caused increased production of endothelin-1, transforming growth factor-β1, vascular endothelial growth factor and augmented extracellular matrix proteins (collagen, fibronectin [FN] and its splice variant extradomain B containing FN), along with evidence of structural alterations, characteristic of diabetes-induced tissue damage. Such changes were prevented by benfotiamine. Furthermore, benfotiamine prevented diabetes-induced oxidative DNA damage and upregulation of p300, a histone acetylator and a transcription coactivator.

Conclusions: Data from the present study suggest that benfotiamine is effective in preventing tissue damage in diabetes and at the transcriptional level such effects are mediated through prevention of p300 upregulation. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2010.00077.x, 2011)

KEY WORDS: Benfotiamine, Diabetic complications, DNA damage

INTRODUCTION

Hyperglycemia in diabetes causes numerous chronic complications affecting microvasculature in the retina, kidney, heart and peripheral nerve. Oxidative stress is an important mechanism causing chronic diabetic complications. Several groups have shown that hyperglycemia initiates increased mitochondrial superoxide production, which damages the electron transport chain leading to accumulation of glycolytic metabolites by inhibiting glyceraldehydes-3-phosphate dehydrogenase[1–3]. Hyperglycemia affects the hexosamine pathway, causes polyol pathway activation, increases advanced glycation end-product formation and activates the diacylglycerol-protein kinase C pathway[4]. At the nuclear level, these pathways increase histone acetylation through p300 and activate transcription factors upregulating gene expression of vasoactive factors and extracellular matrix (ECM) proteins[5,6]. These molecules include, endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), fibronectin (FN) and its splice variant extradomain B containing FN (EDB^+FN), collagen etc[5,6]. It is of further interest that there are regulatory relationships among these vasoactive factors and ECM proteins[7,8]. Among these, ECM protein EDB^+FN is absent in normal mature adult tissues, but is expressed in diabetes[9].

Benfotiamine, a lipid-soluble thiamine derivative, blocks several aforementioned, hyperglycemia-induced pathways of tissue damage in the retina[3] and causes a reduction in superoxide production. Benfotiamine also acts as a direct antioxidant[10]. It has been shown that treatment with benfotiamine prevents functional and structural changes in the retina[3]. Benfotiamine has been shown to have protective effects on non-diabetic vascular injury[11,12]. In a recent study, benfotiamine was shown to prevent cardiac dysfunction in diabetes through modulating PI3 kinase-AKT pathway[12]. Hence, through affecting oxidative stress, a common pathogenetic pathway, benfotiamine might potentially prevent multiple chronic diabetic complications. In contrast, the tissue microenvironment might alter such responses, as local

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milieu influences the pathogenetic process and treatment response in chronic diabetic complications[13]. In keeping with this notion, it has been shown that glucose-exposed vascular cells from various sources respond differently when incubated with benfotiamine[14].

The purpose of the present study was to investigate whether treatment with benfotiamine can prevent diabetes-induced augmented local production of multiple vasoactive factors and ECM proteins responsible for the pathogenesis of chronic diabetic complications. We further investigated the mechanism of action of benfotiamine at the nuclear level.

MATERIALS AND METHODS

Animal Model and Tissue Collection

We carried out the investigations in the streptozotocin (STZ)-induced diabetic rats[5,15,16]. STZ-induced diabetic rat is a well established animal model of type I diabetes, as the drug causes β-islet cell destruction, hypoinsulinemia and hyperglycemia[5,15–17]. The present authors and others have previously shown that these animals develop characteristic biochemical, structural and functional abnormalities affecting the retina, kidney and heart in diabetes[5,13,15,16]. Hence, male Sprague–Dawley rats (Charles River Canada, Senneville, QC, Canada), weighing approximately 200 g, were randomly divided into three groups (n = 10/group): non-diabetic controls (C), diabetic animals (D) and diabetic animals treated with benfotiamine (DB). Diabetes was induced by a single intravenous injection of streptozotocin (STZ; 65 mg/kg in citrate buffer, pH = 5.6), whereas the control animals received the same volume of citrate buffer. Benfotiamine was given (80 mg/kg/day; Doctor's Best, San Clemente, CA, USA) by oral gavage[3]. The dose of benfotiamine was based on previous studies on rats[3,11]. The animals were monitored daily for ketonuria and were given small doses of insulin (1.0 U/day using Linplant; Linshin Canada, Toronto, ON, Canada) to prevent ketosis. The animals were further regularly evaluated with respect to bodyweight and blood glucose concentrations. This time-point was chosen as in our previous studies, we have found that at this time-point, changes characteristic of chronic diabetic complications develop in the retina, heart and kidneys[18,19]. After 4 months of treatment, the animals were killed. Systolic blood pressure was measured by tail plethysmography before the animals were killed, and blood and urine were collected. Blood glucose and serum creatinine were measured. Urine was used for albumin (Nephrat II Albumin ELISA kit; Exocell, Philadelphia, PA, USA) and creatinine measurements (Creatinine Companion; Exocell) following manufacture's instructions, and the albumin creatinine ratios (ACR) were calculated. Retinal, renal and heart tissues were dissected out. Parts of the tissues (retina, left ventricular myocardium and renal cortex) were snap-frozen in liquid nitrogen for gene expression analysis. Small portions of these tissues were fixed in 10% neutral buffered formalin. They were embedded in paraffin for histological and immunocytochemical analysis. All animals were cared for according to the Guiding Principle in the Care and Use of Animals. All experiments were approved by the University of Western Ontario Council on Animal Care Committee.

RNA Extraction and Real-Time RT-PCR

RNA was isolated from the heart, retina and renal cortex as described previously using Trizol reagent (Invitrogen Canada, Burlington, ON, Canada)[5,6]. cDNA was synthesized from RNA The mRNA levels of VEGF, transforming growth factor-β1 (TGF-β1), EDB$^+$FN, FN, collagen type IV alpha 1 (COLα1[IV]) and p300 were quantified using the LightCycle (Roche Diagnostics Canada, Laval, QC, Canada)[5,18,19]. In each reaction tube, the following reagents were added for a final volume of 20 μL: 10 μL of LC DNA Master SYBR Green 1 (Roche Diagnostics Canada), 1.6 μL of 25 mmol/L of MgCl2, 1 μL each of 10 mmol/L forward and reverse primers (Table 1), 5.4 μL of H_2O, and 1 μL of cDNA. The primer sequences are described in Table 1. The mRNA levels were quantified using the standard curve method. Standard curves were constructed by using a serially diluted standard template. ET-1 transcript was quantified by Taqman probe (Applied Biosystems, Foster City, CA, USA), which was designed using primer express v2.0 (Applied Biosystems)[5,18,19].The data was normalized to 18S rRNA to account for differences in reverse transcription efficiencies[5,18,19].

Protein Analysis

Total proteins from rat tissues (retina, heart and renal cortex) were isolated using complete RIPA buffer as previously described[6,7] (NaCl 0.877 g, deoxycholate 1 g, 1 mol/L Tris–HCl pH 7.5 5 mL and 10% sodium dodecyl sulfate 1 mL; volume adjusted to 100 mL using ddH_2O) and protease inhibitors.

Table 1 | Primer sequences for real time RT-PCR

Rat gene	Sequence 5′ → 3′
18S rRNA	GTAACCCGTTGAACCCCATT
	CCATCCAACGGTAGTAGCG
TGF-β	GTAGCTCTTGCCATCGGG
	GAACGTCCCGTCAACTCG
VEGF	GGCCTCCGAAACCATGAACTTTCTGCT
	GCATGCCCTCCTGCCCGGCTCACCGC
ET-1	GCTCCTGCTCCTCCTTGATG
	CTCGCTCTATGTAAGTCATGG
EDB$^+$FN	AGTTAGTTGCGGCAGGAGAAG
	CCGCCATTAATGAGAGTGAT
FN	CCAGGCACTGACTACAAGA
	CATGATACCAGCAAGGAGTT
Collagen αI(IV)	GCCAAGTGTGCATGAGAAGA
	AGCGGGGTGTGTTAGTTACG
p300	GGGACTAACCAATGGTGGTG
	ATTGGGAGAAGTCAAGCCTG

EDB$^+$FN, extradomain B containing fibronectin; ET-1, endothelin-1; FN, fibronectin; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

Protein levels were quantified using Bio-Rad protein assay procedure (Bio-Rad Laboratories, Mississauga, ON, Canada). Collagen was analyzed by western blot using anti-collagen α1(IV) polyclonal antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Detections were carried out by ECL-PLUS kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The blots were quantified by densitometry[6]. For FN quantification, ELISA were carried out using FN ELISA Kit (Millipore Upstate, Temecula, CA, USA) according to the manufacturer's instructions. The developed color was measured at 450 nm wavelength with the Bio-Rad micro plate reader (Bio-Rad Laboratories, Hercules, CA, USA).

Microscopic Analysis

Formalin-fixed tissues were embedded in paraffin. Paraffin-embedded tissues were sectioned (5 μm) and transferred onto positively charged slides. The slides were stained using hematoxylin and eosin. Furthermore, the slides were stained for fibrous tissue using a trichrome stain. A PAS stain was carried out to examine mesangial expansion[18,19].

Immunohistochemistry and Immunofluorescence

Immunocytochemical investigations were carried out using the Vectastain Elite Kit (Vector Laboratories, Burlington, ON, Canada) and monoclonal antibodies against 8-OHdG (8-Hydroxy-2′-deoxyguanosine, 1:50; Japan Institute for the Control of Aging, Fukuroi, Japan) using previously described methodology[19]. The chromagen, 3′-3′ diaminobezine (DAB; Sigma-Aldrich Canada, Oakville, ON, Canada) was used to detect staining showing oxidative damage. Staining with non-immune rabbit serum instead of primary antibodies was used as negative controls. For the detection of histone damage, tissues were stained for phosphorylated histone 2AX (Phospho-H2AX, 1:200; Abcam, Cambridge, MA, USA) and FITC-labeled secondary antibody as previously described[18,19]. Phospho-H2AX is a sensitive marker of nuclear damage. DAPI counter stains were used to locate the nuclei.

Statistical Analysis

The data are expressed at mean ± SEM and were analyzed by ANOVA followed by Student's *t*-test and post-hoc analysis. Statistical differences were considered significant when a *P*-value of <0.05 was obtained.

RESULTS

Diabetes Caused Metabolic Abnormalities in the Animals

Diabetic animals showed hyperglycemia, reduced bodyweight gain and glucosuria. The final bodyweights and blood glucose levels are shown in Table 2. After a further 4 months, diabetic animals showed increased heart weight, as well as heart weight to bodyweight ratio (Table 2). Benfotiamine treatment had no significant effect on blood glucose levels. Although the benfotiamine treated rats showed a mild improvement in bodyweight, they were not significantly different from the controls (Table 2). There were no significant differences in systolic blood pressure between the control and diabetic animals, and benfotiamine treatment had no effect on blood pressure. In contrast, diabetes caused increased albumin excretion and increased albumin creatinine ratios, which were corrected by benfotiamine treatment (Table 2).

Diabetes-Induced Increased Vasoactive Factor Production was Prevented by Benfotiamine

We first examined whether diabetic animals develop upregulation of specific vasoactive factors, characteristic of tissue injury in the target organs affected by chronic diabetic complications. To this extent, we examined three transcripts that are established biomarkers of tissue damage in diabetes[4,5,7,16]. These factors are known to be increased in chronic diabetic complications and mediate tissue damage[4,5,15,16,18]. Hence, chronically diabetic animals were examined after 4 months to examine possible changes in the mRNA expression of ET-1, TGF-β and VEGF. In all tissue examined, mRNA expression of ET-1, VEGF and TGF-β were significantly upregulated. It is, however, of interest to note that the levels of such augmentation were variable. VEGF mRNA upregulation was the highest (∼2.8-fold) in the retina of the diabetic animals followed by the kidneys (∼2.5-fold), and VEGF mRNA upregulation was lowest in the heart (∼1.6-fold). In contrast, although similar levels (∼2-fold) of ET-1 upregulation were seen in the retina and heart, such levels were higher in the kidneys (∼3-fold) in diabetes (Figure 1). The levels of TGF-β mRNA were comparable in all tissues in diabetes. We then examined the effects of benfotiamine on such prevention. Treatment with benfotiamine was more effective in preventing all gene expression in the retina and the heart, bringing them down to close to the normal levels. However, in the kidneys, the efficacy (fold

Table 2 | Clinical monitoring of animals

Groups	Bodyweight (g)	Blood glucose (mmol/L)	Blood pressure (mmHg)	HW (g)	HW/BW (g/kg)	ACR (μg/mg)
Control	621.85 ± 26.99	6.72 ± 0.52	104.5 ± 8.7	1.40 ± 0.07	2.27 ± 0.13	0.15 ± 0.02
Diabetic	504.62 ± 26.50*	24.68 ± 3.03*	109.2 ± 6.8	1.50 ± 0.06	2.77 ± 0.16*	5.24 ± 0.66*
Diabetic + benfotiamine	544.00 ± 16.22	24.95 ± 1.19*	105.6 ± 9.4	1.41 ± 0.02	2.60 ± 0.06**	1.58 ± 0.91

Data are presented as the mean ± SEM. *$P < 0.05$ compared with control for respective parameters, **$P < 0.05$ compared with diabetic for respective parameters, $n = 6$ or more per group. ACR, albumin/creatinine ratio; BW, bodyweight; HW, heart weight.

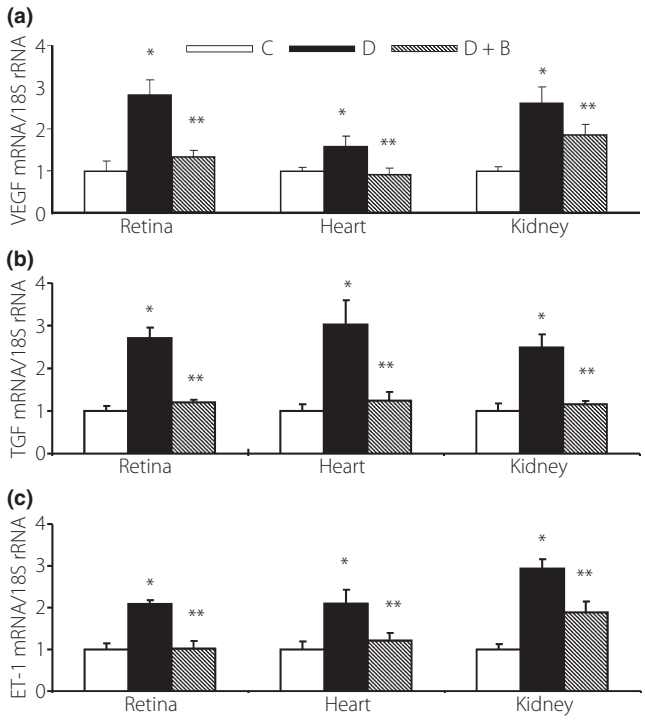

Figure 1 | Upregulation of (a) vascular endothelial growth factor (VEGF), (b) transforming growth factor-β (TGF-β) and (c) endothelin-1 (ET-1) mRNA in the retina, heart and kidneys of the diabetic rats after 4 months of follow up were prevented by treatment with benfotiamine. Data (mean ± SEM) are expressed as a ratio to 18S rRNA, normalized to controls. *P = 0.05 or less from controls, **P = 0.05 or less from diabetics. C, age matched controls; D, diabetics; D + B, diabetics on benfotiamine.

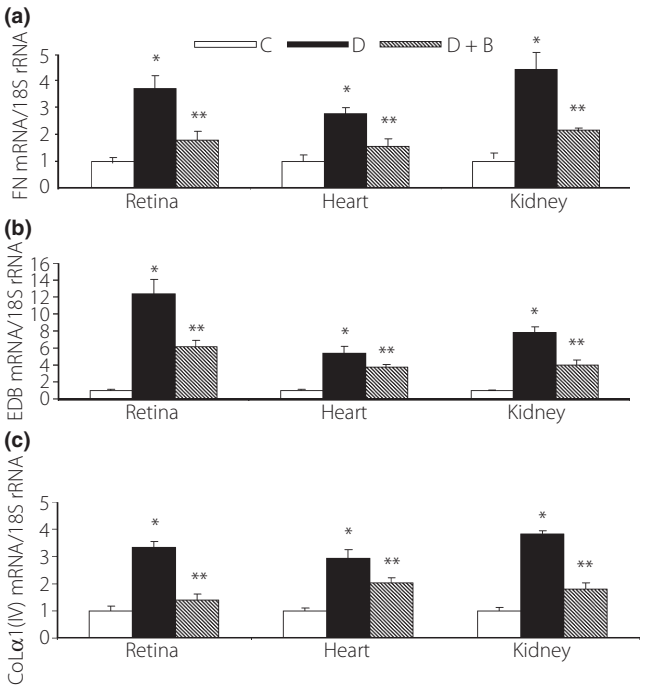

Figure 2 | Upregulation of (a) fibronectin (FN), (b) extradomain B containing FN (EDB$^+$FN) and (c) collagen type IV alpha 1 (COLα1[IV]) mRNA in the retina, heart and kidneys of the diabetic rats after 4 months of follow up was prevented by treatment with benfotiamine. Data (mean ± SEM) are expressed as a ratio to 18S rRNA, normalized to controls. *P = 0.05 or less from controls, **P = 0.05 or less from diabetics. C, age matched controls; D, diabetics; D + B, diabetics on benfotiamine.

reduction) was less pronounced with respect to VEGF and ET-1 mRNA (Figure 1).

Diabetes Induced Augmented ECM Protein Production was Prevented by Benfotiamine

We then expanded our studies and investigated specific ECM proteins of interest in the target organs of chronic diabetic complications. Both ET-1 and TGF-β are known regulators of ECM proteins that are changed in response to diabetic dysmetabolism[4,7,8,15]. Hence, we examined FN, EDB$^+$FN and collagen α1(IV) mRNA. These ECM proteins are increased in all chronic diabetic complications[4–7,15,16,18]. Real-time PCR analysis showed that all three of these transcripts were significantly upregulated in diabetes in all tissues examined (Figure 2). However, similar to the vasoactive factors, there was variability among tissues. FN expressions were more pronounced in the retina (~3.7-fold) and kidneys (>4-fold), and were relatively less increased (~2.7-fold) in the heart. In contrast, EDB$^+$FN upregulation was more robust in the retina (>12-fold) compared with the heart (~5-fold) and kidney (~7-fold). Similarly, Collagen α1(IV) mRNA levels were more pronounced in the kidneys (~3.6-fold) in diabetes compared with others. Benfotiamine treatment prevented augmented expression of all such transcripts (Figure 2). Such preventive effects appeared to be more pronounced (based on the fold reduction) in the retina. To further confirm such ECM protein upregulation, we carried out an assay for FN protein using ELISA. In keeping with the RT-PCR data, FN protein levels were highest in the kidney followed by retina and were least increased (although significant) in the heart (Figure 3). Such increases were prevented by benfotiamine. We further carried out western blots on the renal tissues for collagen α1(IV), as more tissues were available from the kidneys. Diabetes-induced increased renal collagen α1(IV) protein levels were also prevented by treatment with benfotiamine (Figure 3). These data, along with the functional data (e.g. urine albumin, ACR and serum creatinine), show that benfotiamine prevented diabetes-induced renal functional and structural abnormalities (Table 2).

Benfotiamine Prevents Diabetes-Induced Structural Changes in Tissues

Structurally increased ECM proteins, when deposited in the tissue in diabetic complications, manifest as mesangial expansion (a characteristic of early diabetic nephropathy) and focal fibrosis

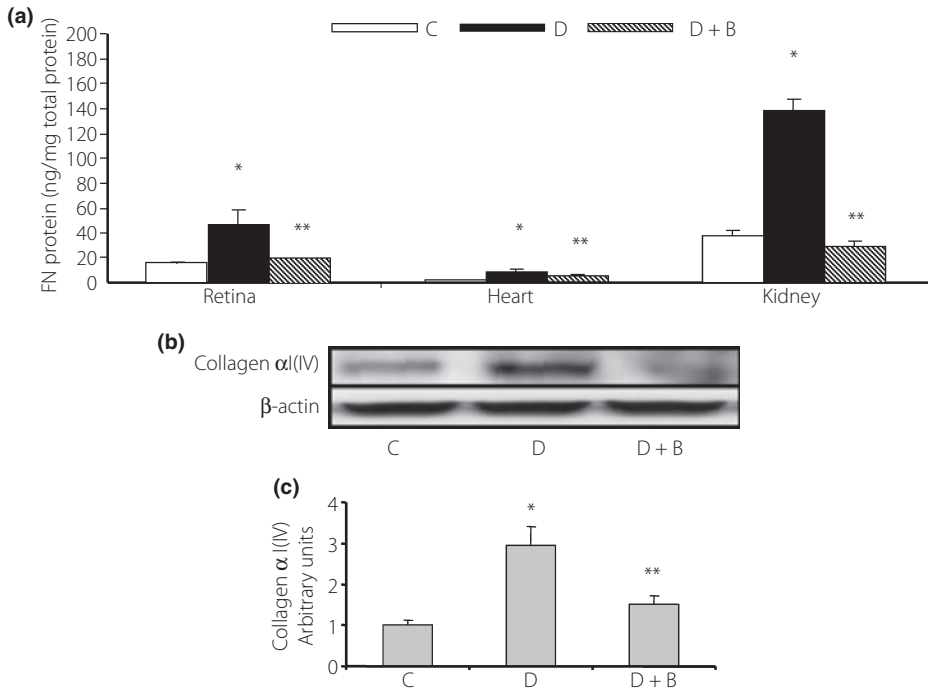

Figure 3 | Upregulation of (a) fibronectin (FN) protein expression (determined by ELISA) in the retina, heart and kidneys of the diabetic rats after 4 months of follow up was prevented by treatment with benfotiamine. (b) Representative western blot and (c) quantitative data of collagen type IV alpha 1 (COLα1[IV]) protein levels in the kidneys of the diabetic rats after 4 months of follow up showing diabetes-induced increased COL1α(IV) protein was prevented by treatment with benfotiamine. Data (mean ± SEM) expressed in relation to total protein (a) or (c) as arbitrary densitometric units, normalized to controls. *P = 0.05 or less from controls, **P = 0.05 or less from diabetics. C, age matched controls; D, diabetics; D + B, diabetics on benfotiamine.

(a feature of diabetic cardiomyopathy). Hence, we examined such parameters microscopically using PAS stain for mesangial expansion and trichrome stains for focal myocardial fibrosis. Chronic diabetes caused mesangial expansion and focal myocardial fibrosis after 4 months (Figure 4). Treatment with benfotiamine prevented such changes.

Benfotiamine Acts by Preventing Diabetes-Induced Oxidative Stress, Oxidative DNA Damage and p300 Upregulation

Diabetes-induced oxidative stress might lead to DNA damage and initiate epigenetic mechanisms, such as p300-mediated acetylation[5,15]. As benfotiamine is a known antioxidant, we proceeded to examine if this is one of the mechanisms by which benfotiamine might prevent development of diabetes-induced changes. Diabetic animals showed increased nuclear stain of 8-OHDG, an established marker for oxidative stress and oxidative DNA damage in all three tissues (Figure 5). We further examined Phospho-H2AX. This is a known marker for oxidative histone damage[19]. Augmented stains of Phospho-H2AX were seen in all examined tissues in diabetes affected animals (Figure 6). Benfotiamine treatment prevented such alteration (Figures 5 and 6). We then examined whether such effects are mediated through nuclear histone acetylator p300 modification. P300 is a known regulator of the majority of the vasoactive factors and ECM proteins under investigation[5,6]. Analysis of p300 mRNA expression showed a diabetes-induced increased p300 mRNA expression in the retina, kidney and in the heart. Benfotiamine treatment prevented such upregulation (Figure 7).

DISCUSSION

In the present study, we have shown upregulation of vasoactive factors and ECM proteins in the retina, heart and kidneys of the diabetic animals, along with augmented oxidative stress and structural damage in these tissues. Such changes were prevented by treatment with the lipid soluble thiamine derivative, benfotiamine. Benfotiamine further prevented renal functional alterations as determined by ACR, a well established marker of diabetic renal damage[20,21]. We have further shown that such action of benfotiamine is mediated by the prevention of oxidative DNA and histone damage, and the activation of transcription coactivator p300.

Benfotiamine has been shown to have vasoprotective effects in endothelial injury caused by various agents[22–24]. In diabetes, benfotiamine has been previously shown to prevent retinal damage[3,23]. It has also been shown to prevent neuropathic and nephropathic changes in diabetes[25,26]. The present authors and

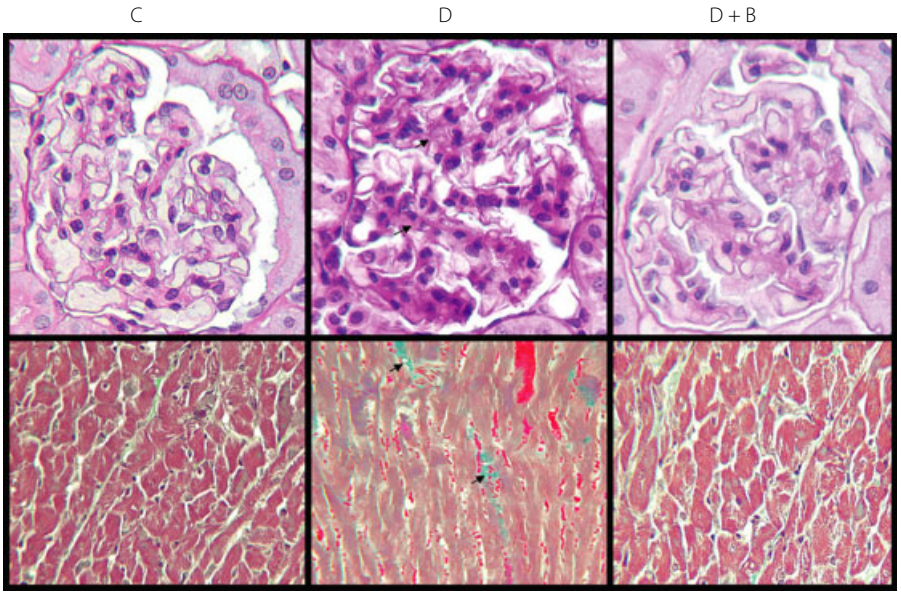

Figure 4 | Photomicrographs showing prevention of (upper panel) diabetes-induced mesangial expansion (PAS stain, arrows) and (lower panel) focal fibrosis (Trichrome stain, arrows) after benfotiamine treatment. C, age matched controls; D, diabetics; D + B, diabetics on benfotiamine.

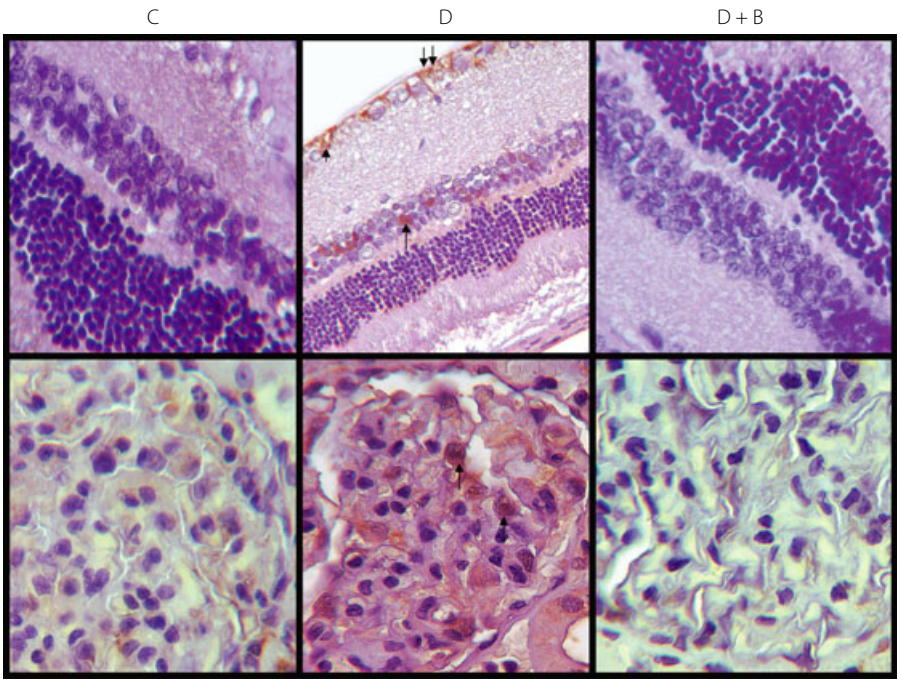

Figure 5 | Photomicrographs showing prevention of (upper panel) diabetes-induced oxidative damage in the retina (8-OHDG stain, arrow shows positive stain in inner nuclear layer, double arrow shows positive stain in ganglion cells, small arrow shows positive stain in endothelium) and kidneys (arrows) after benfotiamine treatment. C, age matched controls; D, diabetics; D + B, diabetics on benfotiamine.

others have previously shown that increased ECM proteins and vasoactive factors, under investigation in the present study, are key mediators of tissue damage in diabetes[15–17,22]. Although these factors appear isolated, in fact, there is extensive inter-dependent regulation among them. For example, both TGF-β and ET-1 are known to regulate FN and EDB$^+$FN in diabetes[7,8]. In contrast, FN, EDB$^+$FN and collagen are capable of sending outside in signaling through integrins leading to VEGF and ET-1 upregulation, and causing endothelial proliferation and differentiation[7,8].

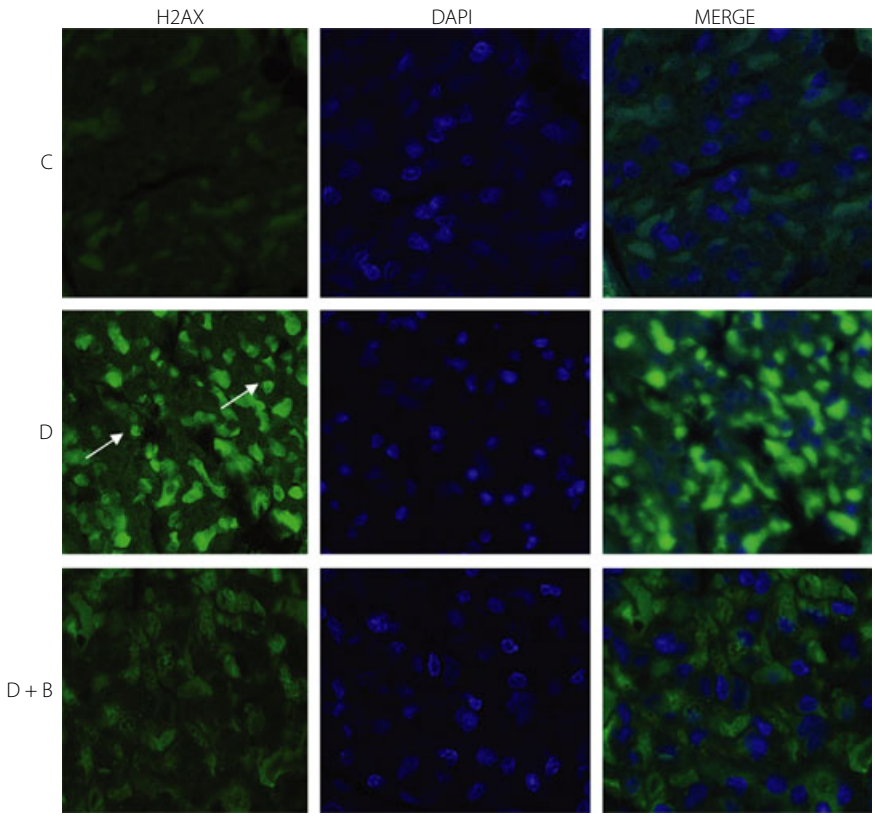

Figure 6 | Photomicrographs of immunofluorescence stains for Phospho-H2AX showing that diabetes-induced oxidative histone damage in the kidneys is prevented by benfotiamine treatment (H2AX positive nuclei are denoted by arrows). C, age matched controls; D, diabetics; D + B, diabetics on benfotiamine; DAPI, nuclear stain.

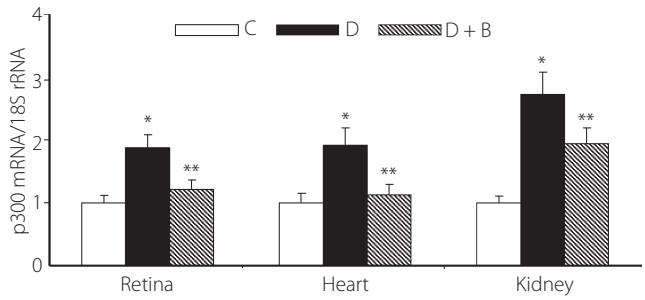

Figure 7 | Upregulation of p300 mRNA in the retina, heart and kidneys of the diabetic rats after 4 months of follow up were prevented by treatment with benfotiamine. Data (mean ± SEM) are expressed as a ratio to 18S rRNA, normalized to controls. *P = 0.05 or less from controls, **P = 0.05 or less from diabetics. C, age matched controls; D, diabetics; D + B, diabetics on benfotiamine.

In the present study, we have found that diabetes causes oxidative DNA and histone damage. We used 8-OHDG and Phospho-H2AX for such analysis. These two are well established markers of oxidative nuclear damage[18,19]. We have previously shown that hyperglycemic nuclear damage activates histone acetylases (HAT) in the nucleus[5,6]. P300 is a well established transcriptional coactivator with HAT activity, regulating multiple transcription factors[5,27,28]. These acetylators are extremely important in the regulation of gene transcription[27–29]. It has been shown that in the absence of transcription coactivators, transcription factors, such as NF-κB, remain silent even after nuclear translocation[29]. It is possible that p300 might represent such a common pathway, which regulates glucose-induced gene transcription at the level of the nucleus through a master switch controlling expression of several transcription factors[6,27]. There are no previous studies directly showing that, mechanistically, benfotiamine acts by preventing DNA damage in the organs affected by chronic diabetic complications. Our data are, however, in keeping with a previous study in which the preventive effects of benfotiamine on angiotensin-induced DNA damage has been shown *in vitro* in the renal cells[10]. The mechanism of benfotiamine's prevention of diabetes induced Phospho-H2AX expression, and p300 activation could possibly be through its preventive effects on oxidative stress. However, possibilities of additional direct effects cannot be excluded and further investigations are needed. Nevertheless, the present study has for the first time shown a possible mechanism of benfotiamine's action by influencing histone acetylation through the prevention of p300 upregulation.

Another interesting phenomenon noted in the present study is that, although statistically significant in all tissues, the amount of upregulation of some molecules of interest was somewhat different in various organs. This was most pronounced in the case of FN. The exact cause of such discrepancies is not known. It is, however, possible that although there are some general similarities, the types of structural and functional changes in various organs are somewhat different in diabetes. In keeping with these results, we have previously shown that the activation of transcription factors, such as NF-κB and AP-1, is a key mediator of glucose-induced increased production of ECM proteins and vasoactive factors[13]. Hence, these findings might suggest the possible additional role of tissue microenvironment, such as blood flow, oxygen tension, metabolism and so on in the pathogenesis of tissue specific damage. However, such notions need further validation by definitive experiments.

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