Tenilsetam prevents early diabetic retinopathy without correcting pericyte loss

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Summary

Hyperglycemia-induced mitochondrial overproduction of reactive oxygen species leads to the activation of different biochemical pathways involved in endothelial damage of the diabetic retina. Tenilsetam [(±)-3-[(2-thienyl)-2-piperazinone] is a dicarbonyl scavenger in the micromolar range and a transition metal ion chelator in the micromolar range. We tested its effect on experimental diabetic retinopathy, and on endothelial cell characteristics in vitro. Streptozotocin diabetic male Wistar rats (60 mg/kg BW) received 50 mg/kg BW tenilsetam (D-T) for 36 weeks, or no treatment (D). The impact of tenilsetam (0–30 mM) on endothelial proliferation, apoptosis, sprouting, cytokine-induced leucocyte-endothelial interaction, and VEGF expression was tested in vitro. Tenilsetam did not affect glycemic control or body weight in diabetic animals. The 3.7 fold increase in acellular capillaries in diabetic rats [p<0.001 vs. non-diabetic controls (N)] was reduced by 70% (p<0.001) through treatment, but pericyte loss (D vs. N ~33%; p<0.001) remained unaffected. In vitro, tenilsetam inhibited endothelial proliferation at lower doses, while inducing apoptosis at high doses. Leucocyte adhesion was only inhibited at high doses. Sprouting angiogenesis of bovine retinal endothelial cells was promoted at lower doses (≤10 mM). At micromolar concentrations, endothelial VEGF expression was upregulated by 100%. Long-term treatment with the AGE-inhibitor and iron-chelating compound tenilsetam inhibits the formation of acellular capillaries without correcting pericyte loss. The compound has dose-dependent effects on endothelial cell function. These data suggest that, independent of known properties, tenilsetam shows important rescue functions on endothelial cells which could be useful for the treatment of early diabetic retinopathy.

Keywords

Experimental diabetic retinopathy, pericytes, endothelial survival, VEGF, tenilsetam

Introduction

Retinopathy is the most prevalent microvascular damage in diabetes mellitus. The progressive occlusion of retinal capillaries represents the cell-biological substrate of chronic hyperglycemic damage. Histologically, diabetic retinal capillary lesions start with the loss of pericytes (1–3) followed by the formation of non-perfused acellular capillaries, which represent the paradigm lesion of incipient diabetic retinopathy, both, in humans, and in animal models. Pericytes protect capillary endothelial cells from regressive signals during vessel development and inhibit extensive proliferation of endothelial cells (4). The cause of early loss of pericytes during incipient diabetic retinopathy is unclear. Some favour the hypothesis that pericyte loss is the result of cellular damage and apoptosis, while we demonstrated earlier that pericytes may be eliminated through an active process involving the angiopoietin-Tie system (5). The underlying cause of this cell biological sequence of events initiates with chronic hyperglycemic...
mia (6, 7). Several downstream mechanisms have been proposed to mediate tissue damage, including increased polyol pathway flux (8), de novo DAG synthesis with resultant activation of PKC-isoforms (9), increased hexosamine pathway flux (10) and increased formation of advanced glycation endproducts (AGEs) (11, 12). Inhibitors of each of the pathways have been found to exert their effects by other mechanisms. For example, aminoguanidine not only inhibits the formation of AGEs, but is also an inhibitor of the PKC (14) the nitric oxide synthase (15, 16), aldose reductase (17) and possesses antioxidative properties (18). Since ROS overproduction is a crucial initiator of vascular complications, and oxidative stress is consistently demonstrated in retinae from diabetic origin, antioxidants would be a candidate group for treatment. Antioxidants such as vitamin C/E or nicotinamide, who work stochiometrically, were either not successful in preventing diabetic retinopathy (19, 20) or work only in combination with many other compounds (21). Baynes, et al. detected that several AGE-inhibitors, whose primary mode of action is carbonyl trapping, are strong antioxidants (22). Furthermore, trapping transition metals reduces metal-catalyzed oxidative stress to proteins. Tenilsetam [(±)-3-(2-thienyl)-2-piperazinone] – a compound developed to reduce dementia in Alzheimer patients – inhibits AGE-formation in vitro and in vivo at high doses, presumably by carbonyl trapping (23, 24). At much lower concentrations, tenilsetam shows strong copper chelating activity (22), suggesting dual beneficial effects on important mechanisms of vascular damage in diabetes. Tenilsetam administration at a dose of 50 mg/kg body weight/day in diabetic Sprague-Dawley rats over 4 months prevented the development of lens opacity (23).

We tested the effect of long-term administration of tenilsetam in a rat model of diabetic retinopathy. Surprisingly, we identified for the first time that a compound had a beneficial effect on the formation of acellular capillaries without correction of pericyte dropout. We therefore assessed the effect of tenilsetam on basic endothelial functions in vitro, such as survival, apoptosis, sprouting, leukocyte-endothelial interactions, and expression of VEGF.

Materials and methods

Materials

Experiments performed in this study adhered to the Association for Research in Vision and Ophthalmology (ARVO) statement for the “Use of Animals in Ophthalmic and Vision Research”.

Tenilsetam [(±)-3-(2-thienyl)-2-piperazinone] was kindly provided by Dr. Ursula Schindler (Sanofi-Aventis, Frankfurt, Germany). Pepsin and trypsin were obtained from Merck (Darmstadt, Germany). Trypsin-EDTA was from Invitrogen GmbH (Karlsruhe, Germany). Streptozotocin, D(−)-glucose, gelatine, collagen type I, bovine serum albumin (BSA), paraformaldehyde and crystal violet were purchased from Sigma (Munich, Germany). Endothelial cell basal medium (ECBM) and endothelial cell supplement pack were from PromoCell (Heidelberg, Germany), fetal calf serum (FCS) was from BioWest (Nuaille, France), and cell culture plates were form Nunc GmbH (Wiesbaden, Germany). Photometric detection was performed by a microtiter plate reader from Molecular Devices (Munich, Germany).

Experimental diabetic retinopathy

Six-week-old male Wistar rats (Charles River, Sulzfeld, Germany) weighing 271 ± 15.3 g were rendered diabetic by injection of 60 mg/kg body weight streptozotocin in 0.05 M sodium citrate, pH 4.5. Glucose levels and body weight were monitored consecutively and glycated haemoglobin was determined by affinity chromatography (Glyc Affin, Isolab, Akron, OH, USA). Rats were grouped as diabetic when blood glucose exceeded 15 mM at 1 week after induction. Diabetic animals were randomly assigned to receive tenilsetam at a dose of 50 mg/kg body weight per day in drinking water or no treatment.

Retinal vascular preparations were performed as described (12, 25) and acellular capillaries and pericyte numbers were determined by two observers blinded to the identity of the samples. Tenilsetam concentration in the serum of treated diabetic animal was measured by HPLC exactly as described (26).

Cell culture and media

Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cord vein by collagenase treatment as described previously (27) and grown in endothelial cell basal medium (ECBM) containing growth factors (hEGF and bFGF), hydrocortisone, gentamicinsulfate and amphotericin B (all from PromoCell-supplement pack) and 5% FCS. HUVECs were cultured in 5.5 mM glucose and used between passages 3 and 5.

Bovine retinal endothelial cells (BRECs) were isolated as described (28) and cultured in ECBM with 10% FCS, containing the same supplements as described for HUVEC-cell medium with addition of 0.1% of ECGS (PromoCell). For sprouting assay, passages 19 and 20 were used.

Bovine aortic endothelial cells were kindly provided by Dr. Angelika Bierhaus (Medical Department, University of Heidelberg, Germany), and were cultured in DMEM, containing 10% FCS, antibiotics, and essential amino acids (29).

BrdU assay

Endothelial cell proliferation was determined by the amount of the incorporated pyrimidine analogue 5-bromo-2'-deoxyuridine (BrdU) using the cell proliferation BrdU-ELISA kit from Roche (Mannheim, Germany) according to the manufacturer’s instructions. Briefly, HUVECs were seeded in 96-well microtiter plates (5,000 cells/well) and cultured three days in complete ECBM (5% FCS). Then, cells were starved in ECBM without FCS or other growth factors. After 24 h, different concentrations of tenilsetam (0–30 mM in absence or presence of VEGF (20 ng/ml) diluted in ECBM with 0.05% FCS) were added for 24 h. After 18 h, BrdU labeling solution was added for the remaining 6 h. Finally, labeling medium was removed, cells were fixed with FixDenat for 30 min and subsequently blocked with 3% BSA in PBS overnight at 4°C. Cells were washed and incubated with anti-BrdU-POD for 90 min at room temperature. Cells were
rinsed three times, followed by substrate addition, incubation for 30 min in the dark, and photometric detection (450 – 650 nm).

**Cell proliferation assay**
For cell proliferation assays, HUVECs were seeded in 96-well culture plates (5,000 cells/well). Cells were cultured for three days, then normal medium was replaced by ECBM with either 0.2% or 10% FCS in presence or absence of tenilsetam (0–30 mM). After an incubation period of 48 h, cells were fixed with methanol/acetone, stained with crystal violet and cell numbers were quantitated by measuring optical density using an ELISA reader (OD<sub>590</sub>). At least three wells were prepared in parallel for each condition.

**Apoptosis in cultured HUVECs**
Apoptosis of HUVECs was analysed by cell staining using the Annexin V-FITC kit according to the manufacturer’s instructions (Immunotech, Marseille, France). Briefly, after treatment of cells with different concentrations of tenilsetam for 18 h, floating as well as adhesive cells were collected and washed once in cold medium at 180 x g for 5 min. The cell pellet was resuspended in 100 µl cold binding buffer, containing 1 µl of annexin V-FITC solution and 5 µl dissolved propidium iodid. After 10 min incubation in the dark, 400 µl of cold binding buffer was added, and the percentages of apoptotic and necrotic cells was evaluated by flow cytometry (Epics<sup>®</sup> XL with software Expo32 ADC from Beckman-Coulters, Krefeld, Germany). Positive controls were obtained by treatment of cells with 1 µM staurosporine (Merck Biosciences GmbH, Darmstadt, Germany) for 4 h or by maintaining HUVECs in suspension, seeding them on BSA-coated plates for 18 h.

**Endothelial sprouting assay**
The effect of tenilsetam on endothelial cell sprouting was investigated using the microcarrier-based fibrin gel angiogenesis assay described by Nehls and Drenckhahn with modifications as described below (30). Bovine retinal endothelial cells (BREC) were trypsinized and allowed to adhere onto gelatine-coated microcarrier beads (MC) „cytodex 3“ (Amersham Biosciences, Freiburg, Germany). Cells were grown to confluence for 48 h. A fibrinogen stock solution (Merck Biosciences GmbH, Darmstadt, Germany) was diluted to 1.8 mg/ml in Dulbeccos PBS, pH 7.4 (Invitrogen GmbH, Karlsruhe, Germany) containing 200 U/ml aprotinin (Bayer, München, Germany) as protease inhibitor. Sixty µl of cell-coated MC were suspended in 600 µl of the fibrinogen solution and 0.65 µl thrombin was added to induce polymerisation. After polymerisation (20 min at 37°C), gels were equilibrated in endothelial cell medium with 0.5% FCS. After 2 h at 37°C, medium was replaced by ECBM with tenilsetam (0–30 mM) containing 0.5% FCS. Basic FGF (2 ng/ml) was added as a positive control. The angiogenic response was quantified 48 h later by determination of the average number of sprout formation per MC. For each condition 50 MC were analysed.

**Leucocyte-endothelial interaction**
Adhesion of THP-1 myelomonocytic cells to cultured monolayers of HUVEC was tested as previously described (31, 32).

Briefly, HUVEC were grown to confluence onto 96-well plates. Sixteen hours prior to the adhesion assay, medium was changed to 0.2% FCS DMEM without or with TNF-α (10 ng/ml, R&D Systems, Wiesbaden, Germany). After this incubation period, HUVEC were washed twice with serum-free medium before addition of THP-1 cells. Fluorescence labelled differentiated THP-1 cells were washed twice followed by no pretreatment or by stimulation with phorbol myristate acetate (PMA; 50 ng/ml). Cells were washed and added to HUVEC (100,000 cells/well) at 37°C for 60 min in the absence or presence of inhibitors, as indicated in the figure legends. After washing, adhesion of THP-1 cells was quantified as the percentage of total cells added using a fluorescence microplate reader (Bio-Tek, Neufahrn, Germany).

**VEGF expression**
BAE were incubated for 2 hours with increasing concentrations of tenilsetam (0.025–100 mM), and VEGF expression was assessed using RT-PCR. VEGF and β-actin mRNAs were analysed by RT-PCR. Total RNA was extracted from BAE cells. The RNA concentration was spectrophotometrically determined. Following DNase I treatment, first-strand cDNA was synthesised using 250 ng random hexamers, 250 ng oligo(dT), 500 ng total RNA and 200 units of reverse transcriptase Super Script<sup>™</sup> II (Invitrogen, Germany) according to the manufacturer’s instructions. VEGF cDNA fragment was amplified by PCR, using forward primer (5'-AATGCTTTCTCCGCTC –3') and reverse primer (5'-AATGCTTTCTCCGCTC –3'). The forward primer oligonucleotide and the reverse primer oligonucleotide correspond to the

**Figure 1: Influence of tenilsetam on retinal morphology.**
Acellular capillaries (panel A) and pericyte counts (panel B) in non-diabetic (N), diabetic (D) and diabetic animals treated with tenilsetam (D + T) were assessed by quantitative retinal morphometry. Shown are numbers of acellular capillaries and pericytes per mm² of retinal area. *P < 0.001 vs. N, **P < 0.05 vs. D (n = 7–9 animals/group).
nucleotide sequences 1–17 and 525–540 of mouse VEGFm RNA (GenBank accession no. M95200). The β-actin DNA fragment was amplified using the forward primer (5′-AGAGGTATCGACCTGAAGTACC-3′) and the reverse primer (5′-CCACCAAGACAACACTGTGTTGGCAT-3′) that correspond to 106–130 and 803–827 nucleotide sequences of the mouse gene, respectively (GenBank accession No. X03765). The amplification was performed using HotStarTaq DNA Polymerase with 2 mM final concentration of MgCl₂ according to the manufacturer’s protocol. Amplified PCR products were analysed by electrophoresis in 1% agarose gel. Gels were photographed using the Intas digital camera and Intas GDS Application software (Intas, Science Imaging Instruments GmbH, Gottingen, Germany). The bands corresponding to VEGF mRNAs were measured by analysis software (Olympus, Germany) and were normalized to β-actin of the corresponding cDNAs.

Statistical analysis
Results are given as mean ± SD unless otherwise stated. For statistical analysis, analysis of variance (ANOVA) and the Bonferroni multiple comparison test were used (Instat; GraphPad, San Diago, CA, USA).

Results
Streptozotocin-induction of diabetes resulted in stable hyperglycaemia and progressive weight loss. After 36 weeks of diabetes, there was no significant difference between the mean blood glucose level of the non-treated rats and the tenilsetam-treated rats (D 29.90 ± 2.7 mM vs. D+T 30.16 ± 4.5 mM). Mean blood glucose of the control group was 6.80 ± 0.9 mM. Tenilsetam treatment had no effect on the weight gain of the diabetic group (D 352 ± 76 g vs. D+T 316 ± 69 g; N 660 ± 138 g; HbA1 5.1 ± 0.6%). Metabolic control as determined by glycated haemoglobin in diabetic rats (D 16.2 ± 1.6% vs. N 5.1 ± 0.6%; p<0.001) was not altered by tenilsetam (D+T 18.0 ± 2.1%). Oral administration resulted in a significant serum level of tenilsetam [1.01 ± 0.63 µg/ml (5.54 ± 3.46 nM)] as measured by HPLC.

Retinal digest preparations revealed a 3.7-fold increase in acellular, occluded capillaries in diabetic animals compared to the control group (D 66.3 ± 23.6 vs. N 17.9 ± 12.2) (Fig. 1A). Retinas from tenilsetam-treated rats had 70% less acellular cap-
illaries (D+T 32.0 ± 6.6) than untreated diabetic rats. Diabetes caused a significant, 33% reduction of pericyte numbers, compared with non-diabetic control animals (D 1390 ± 220 pericytes/mm² of capillary area; N 2083 ± 359 pericytes/mm² of capillary area; p<0.001). Tenilsetam had no significant effect on pericyte loss (D+T 1513 ± 251; p n.s. vs. D) (Fig. 1B).

Based on these findings, we aimed to assess possible endothelial survival promoting functions of tenilsetam in vitro. First, we analysed, using standard proliferation assays, whether tenilsetam affected endothelial cell proliferation. As depicted in Figures 2 and 3, tenilsetam inhibited proliferation of HUVECs in vitro only at high concentrations. Cell numbers were reduced by 14.1%, 35.5% and 53.6% after 24 h of incubation with 3, 10 and 30 mM of tenilsetam, respectively (Fig. 3A). Consistently, basal DNA-synthesis was reduced by 24.1%, 28.2% and 90.8% when HUVECs were incubated for 24 h with 3, 10 or 30 mM tenilsetam as analysed by BrdU-assay. Addition of 10% FCS to the growth medium intensified the inhibitory effect of tenilsetam on endothelial proliferation (Fig. 3B). Furthermore, cell morphology after 4 days of tenilsetam treatment revealed that HUVECs were spreading with increasing tenilsetam concentrations, indicating a transition from a proliferating into a resting and adhesive cellular phenotype (Fig. 2). The altered phenotype was not associated with major changes of migratory or adhesive properties of endothelial cells in quantitative tests. We observed a minor increase in migration towards a chemotactic agent in the presence of tenilsetam (data not shown).

Figure 4: Induction of apoptosis by tenilsetam. HUVEC were incubated for 18 h in the absence (−) or presence of different concentrations of tenilsetam as indicated. As positive controls, apoptosis was induced by treatment of cells with 1 µM staurosporine (Stau) for 4 h or by seeding the cells on BSA-coated plates (BSA) for 18 h to prevent adhesion. Apoptosis was detected by cell staining with Annexin V-FITC and subsequent FACS-analysis. Experiments were performed in duplicate for each condition, and mean values of a representative experiment out of three are shown.

We then analysed the induction of apoptosis of endothelial cells and the possible impact of tenilsetam. As depicted in Figure 4, low concentrations of tenilsetam had no effect on endothelial cell apoptosis. High doses (30 mM) of tenilsetam induced a moderate increase of apoptotic cells.

We tested the function of tenilsetam in an assay in which a monolayer of endothelial cells was cultured on microcarrier beads and embedded in three-dimensional fibrin gel. This assay pictures various endothelial functions such as activation, migration, proliferation, and tube formation of endothelial cells. In our context, it allows to discriminate whether tenilsetam is pro- or antiangiogenic. Tenilsetam dose-dependently increased spraying of microvascular endothelial cells by up to 60%. At very high doses, tenilsetam inhibited spraying to basal levels (Fig. 5).

Since tenilsetam affects inflammation (33), we tested the possible interference with leucocyte-endothelial interaction. HUVEC monolayers were prestimulated with TNF, and THP-1 myelomonocytic cells were tested for their adhesiveness. Low doses of tenilsetam did not affect the endothelium-leucocyte interaction, while high doses (above 10 mM) significantly inhibited the adhesion (Fig. 6).

Hypoxia-inducible factor 1α (HIF 1α) controls VEGF transcription, and is inactivated by HIF prolyl hydroxylases under normoxia. Since Fe (II) is at the active centre of this class of dioxygenases, hypoxia, and Fe-chelators stabilize HIF through inactivation of the hydroxylases. We therefore exposed endothelial cells to increasing concentrations of tenilsetam, and measured VEGF transcription by RT-PCR. VEGF expression was doubled already at doses of 25 µM of tenilsetam (Fig. 7). Consistent with the proposed mechanism, we did not observe a dose-dependent effect.

Discussion

Long-term administration of tenilsetam results in a significant reduction of acellular capillaries without restoration of pericyte loss in the diabetic rat retina. The in vitro data suggest that tenilsetam exhibits a dose-dependent dual effect on endothelial cells. While low doses support endothelial sprouting and survival, high doses are anti-angiogenic, and moderately pro-apoptotic. Only high doses, not achieved in our model, interfere with the interaction of endothelial cells and leucocytes. However, low doses of tenilsetam which we achieved in vivo induce VEGF up-regulation in endothelial cells in vitro. Thus, we conclude that the
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Figure 6: Influence of tenilsetam on leukocyte adhesion. PMA-stimulated adhesion of THP-1 cells to cultured endothelial cells that had been left unstimulated or that were stimulated for 16 h with TNF-α (7) is shown in the absence or presence of blocking mAb against CD18, CD11b, or ICAM-1 (each at 20 µg/ml) as well as in the presence of increasing concentrations of tenilsetam as indicated. The degree of cell adhesion is shown as percentage of adherent cell (mean ± SD of a typical experiment out of three).

Figure 7: Influence of tenilsetam on endothelial VEGF transcription. BAE cells were stimulated for 2 h with increasing concentrations of tenilsetam as indicated, and VEGF expression was assessed by RT-PCR. * p<0.01 vs. Control.

In vivo effects observed in our animal model are based on prosurvival stimuli which may be linked to the chelating properties of the compound.

This is the first observation that endothelial cells partly survive exposure to the diabetic environment without a correction of pericyte loss. Glucose normalisation and AGE inhibition have been demonstrated to correct the formation of acellular capillaries (representing survival of endothelial cells) and pericyte loss (12, 13). It is undisputed that pericytes support endothelial survival as demonstrated in genetically modified mice (34). Mice with a heterozygous deletion of the pericyte attracting ligand and PDGF-B have more acellular capillaries when they have less pericyte recruitment to the endothelium (35, 36). The endothelial damage is aggravated during chronic hyperglycaemia, suggesting that this paradigm holds true also under stress conditions such as diabetes. Pericyte recruitment is further regulated by the balance between Angiopoietin-1 and -2 acting on Tie-2. Diabetic pericyte loss is prevented when Ang-2 gene dose is reduced by half (5). Even when the pericyte protection for endothelial cells is maintained, the formation of acellular capillaries is not completely prevented. Thus, in the context of the present findings, it can be concluded that tenilsetam provides a prosurvival set of signals which compensates for the absence of pericyte protection.

A significant finding of our study is that tenilsetam exerts beneficial effects on endothelial cells, and that this effect is dose-dependent. While low-dose tenilsetam supports endothelial sprouting, high doses are anti-angiogenic, and pro-apoptotic in vitro. In vitro sprouting is determined by reprogramming of endothelial cells towards invasion, migration, proliferation, and tube formation (30). At present, it is not clear which of the specific steps of the sprouting process are mostly affected by tenilsetam. In this context, it is important to reconsider the pharmacological properties of tenilsetam. Shoda, et al., using high levels of tenilsetam (in the 100 mM range), reported findings that categorised tenilsetam as an AGE inhibitor (33). Subsequent studies with similar doses found that, due to the carbonyl-scavenging attributes, tenilsetam protects against the neurotoxic effects of the glycolytic intermediate and AGE precursor methylglyoxal (MG) (37). MG is also particularly involved in the endothelial-damaging early formation of intracellular AGEs in the diabetic retina, and its rapid and sustained detoxification by overexpression of the detoxifying enzyme glyoxalase is a promising new approach to reduce hyperglycaemia-promoted vascular damage (38). From the experiments of the present study, we cannot address this question, because methylglyoxal-type AGE were not evaluated. However, given the comparatively low level of tenilsetam in vivo, the primary mode of action in the in vivo experiments appears different from AGE-inhibition. In fact, tenilsetam has different actions at different concentrations in vivo. Price, et al. found that low-dose tenilsetam is a strong transition-metal-chelater, suggesting that it may act by reducing metal-catalyzed oxidative damage to tissues (32). Of note, by comparison with classical antioxidants, there are substantial differences in the effects on the relevant parameters in the retinopathy model: antioxidants partially preserve pericyte loss while having no, or marginal, effects on the formation of acellular capillaries, while tenilsetam yielded the opposite results (24). Catalytic antioxidants like R-alpha-lipoic acid prevent both, pericyte loss and the formation of acellular capillaries (39). Iron is a critical component of the complex translating hypoxia into the expression of hypoxia-induced growth factors such as VEGF. Hypoxia-inducible factor (HIF) hydroxylases are all Fe (II)- and 2-oxoglutarate-dependent dioxygenases mediating tissue demand for oxygen with the transcription of factors initiating angiogenesis (40, 41). This explains why iron chelators induce the transcription of VEGF. VEGF not only induces retinal neovascularization, but serves as survival factor for the retinal capillaries. We have demonstrated that VEGF is up-regulated in the early diabetic retina, and speculated that VEGF serves as a survival factor for retinal tissue cells in diabetes (42). More recently, we found that mice with a conditional inactivation of VEGF in neural tissue (including the retina) develop a retinopathy-like phenotype, further supporting this concept (43). The present data build further on this, because we found improved endothelial cell survival in the retina in hyperglycaemia.
Recently, several groups have reported that leucocyte adherence to the diabetic endothelium and subsequent damage is an important mechanism of retinopathy propagation (44, 45). Tenilsetam had an effect on the inflammatory interaction of leucocytes with endothelial cells in our study. However, again, this was only achieved at very high doses, which are not achieved in vivo. Thus, a positive effect on experimental retinopathy at a dose not affecting leucocyte-endothelial interaction makes a proinflammatory component of diabetic retinopathy on the leucocyte-endothelial level unlikely.

In summary, our study demonstrates that i) prevention of pericyte loss is not a major component of the rescue program for diabetic retinal capillaries, ii) transition-metal-chelating compounds are candidate drugs for diabetic complications, and iii) direct survival promoting effects on vascular cells besides growth factor-induced effects should be considered. Thus, compounds such as tenilsetam that share the propensity of promoting endothelial cell survival in the diabetic retina may be considered as potential new treatments in early diabetic retinopathy.

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