Poly(ADP-Ribose) Polymerase Is Involved in the Development of Diabetic Retinopathy via Regulation of Nuclear Factor-κB

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The current study investigated the role of poly(ADP-ribose) polymerase (PARP) in the development of diabetic retinopathy. Activity of PARP was increased in whole retina and in endothelial cells and pericytes of diabetic rats. Administration of PJ-34 (a potent PARP inhibitor) for 9 months to diabetic rats significantly inhibited the diabetes-induced death of retinal microvascular cells and the development of early lesions of diabetic retinopathy, including acellular capillaries and pericyte ghosts. To further investigate how PARP activation leads to cell death in diabetes, we investigated the possibility that PARP acts as a coactivator of nuclear factor-κB (NF-κB) in the retinal cells. In bovine retinal endothelial cells (BRECs), PARP interacted directly with both subunits of NF-κB (p50 and p65). More PARP was complexed to the p50 subunit in elevated glucose concentration (25 mmol/l) than at 5 mmol/l glucose. PJ-34 blocked the hyperglycemia-induced increase in NF-κB activation in BRECs. PJ-34 also inhibited diabetes-induced increase expression of intercellular adhesion molecule-1, a product of NF-κB–dependent transcription in retina, and subsequent leukostasis. Inhibition of PARP or NF-κB inhibited the hyperglycemia (25 mmol/l glucose)-induced cell death in retinal endothelial cells. Thus, PARP activation plays an important role in the diabetes-induced death of retinal capillary cells, at least in part via its regulation of NF-κB. Diabetes 53:2960–2967, 2004

Poly(ADP-ribose) polymerase (PARP) is a nuclear enzyme that is involved in the cellular response to DNA injury (1). DNA breaks are believed to be obligatory triggers for the activation of PARP. These DNA breaks are induced by a variety of environmental stimuli, such as oxidative and nitrosative stress. Upon encountering DNA strand breaks, PARP catalyzes the cleavage of NAD+ into nicotinamide and ADP-ribose, and then uses the latter to synthesize polymers of ADP-ribose, covalently attached to nuclear proteins, including PARP itself. When DNA damage is mild, poly(ADP-ribose)ylation facilitates cell survival. When DNA damage is severe, PARP activation can induce cellular energetic disturbances, leading to cell dysfunction or death (2,3). Genetic disruption of PARP or pharmacologic inhibition of this enzyme has beneficial effects on inflammation, shock, stroke, myocardial ischemia/reperfusion, and prevents the onset of autoimmune diabetes (2–7).

PARP is now recognized to play a role also in the regulation of gene transcription. Several transcription factors, including nuclear factor-κB (NF-κB) (8,9), p53 (10, 11), and AP-1 (12), interact with PARP and are regulated by it. By using PARP inhibitors or knocking out PARP gene in cells or mice, both NF-κB activation and transcription of NF-κB–dependent genes, such as inducible nitric oxide synthase or intracellular adhesion molecule (ICAM)-1, can be reduced (9,13–15), suggesting that inhibition of poly(ADP-ribose)ylation might prevent the consequences of inflammation or stress by modification of NF-κB–dependent pathways.

Evidence suggests that oxidative and nitrosative stress are greater than normal in retinas from diabetic animals (16–18), thus potentially activating PARP and contributing to the pathogenesis of diabetic retinopathy. In this study, we demonstrate that PARP activity in retina is increased in diabetes and that inhibition of PARP inhibits the development of early lesions of diabetic retinopathy. These beneficial effects of PARP inhibition are mediated at least in part via its regulation of NF-κB.

RESEARCH DESIGN AND METHODS

Type 1 diabetes was induced in fasted male rats (Lewis) with streptozotocin (60 mg/kg body wt), and animals were housed in ventilated microisolator cages. All experiments followed the guidelines set forth by the Association for Research in Vision and Ophthalmology Resolution on Treatment of Animals in Research. Insulin was given as needed to maintain body weight and allow a slow increase in body weight while allowing hyperglycemia, polyuria, and hyperphagia (0–2 units every 2–3 days). Hyperglycemia was estimated every 2–3 months by assay of GHb using a Variant kit (Bio-Rad, Hercules, CA) and by assay of blood glucose concentration. One week after the injection of streptozotocin, diabetic rats were randomized to receive the PARP inhibitor PJ-34 (the hydrochloride salt of N-[3-oxo-5,6-dihydro-phenanthridin-2-yl]-N, N-dimethylacetamide; 20 mg/kg body wt daily in food) or to remain as diabetic controls. PJ-34 is based on a modified phenanthridine structure and is ~10,000 times more potent than the prototypical PARP inhibitors nicotinamide and 3-amino benzamide (19). Diabetic rats and age-matched nondiabetic controls were killed at 12 and 36 weeks of treatment.

Cultured bovine retinal endothelial cells. Primary cultures of bovine retinal endothelial cells (BRECs) were established after isolating the cells...
from fresh bovine eyes by homogenization and a series of filtration steps as described previously (20). A cell sorter was used to further purify the primary BRECs by using DiI-Ac-LDL (Biological Technologies, Stoughton, MA) uptake, and cell purity was confirmed by staining with factor VIII (Dako, Carpinteria, CA). BRECs were subsequently cultured in 5 mM d-glucose with endothelial cell basal medium (Clonetics, Walkersville, MD) supplemented with 10% PBS-pretreated fetal bovine serum, 50 μg/ml heparin (Sigma, St. Louis, MO), bovine brain extract (Clonetics), and 50 μg/ml endothelial cell growth factor (Clonetics). Only passage 3–6 BRECs were used in experiments. When cell populations reached 60–70% confluence, the concentration of horse serum was decreased to 2% to reduce the growth rate of the cells. Then, the cultures were incubated in 5 or 25 mM d-glucose. Cells in 25 mM d-glucose were treated with or without different doses of PJ-34 or SN-50 (Calbiochem, San Diego, CA). Cells were cultured at 37°C in 5% CO2 and 95% air, and the media were changed every other day.

Isolation of retinal blood vessels. The retinal vasculature was isolated by two different methods because of different sensitivities of antibodies used for immunohistochemistry to fixation.

Osmotic shock method. Freshly isolated retinas from rats were incubated in distilled water (high-performance liquid chromatography grade) for 1 h, followed by brief exposure (2 min) to DNase 1 (2 mg/ml). Retinal vasculature was isolated under microscopy by repetitive inspiration and ejection through Pasteur pipette (3–5 mm diameter). Retinal vasculature isolated by this method showed a normal complement of nuclei and were devoid of nonvascular materials (21). The retinal vasculatures were laid out on glass slides and air dried for immunohistochemistry of PARP activity. TUNEL assay in vivo. Rat retinal vasculatures were isolated as described by us previously (22,23). Briefly, freshly isolated eyes were fixed with 10% neutral buffered formalin. Retinas were isolated, washed in water overnight, and then incubated with 3% Difco crude trypsin (BD Biosciences, Sparks, MD) at 37°C for 1 h. Nonvascular cells were gently brushed away from the vasculature, and the isolated vasculatures were used for p50 immunostaining, terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) assay, and assessment of pathology.

Immunohistochemistry on paraffin sections and isolated retinal microvasculature. Polyclonal antibodies were used to detect p50 (Santa Cruz Biotechnology, CA), p65 (Santa Cruz Biotechnology, CA), and PARP (Santa Cruz Biotechnology, CA). Blood was removed from the eyes by a single incision. Eyes were placed in 5% formaldehyde (Sigma, St. Louis, MO) and kept in that solution for 24 h. After 24 h, the eyes were sliced into 150-micron-thick frozen sections using a cryostat. Sections were then transferred to 2% formaldehyde in PBS for 5 min, washed in PBS, and rinsed with PBS. After centrifugation (3,500 × g), the supernatant was removed, and the samples were incubated with various antibodies at 37°C for 1 h. Primary antibodies were then removed, and samples were washed three times with PBS. Sections were then incubated with a biotinylated anti-mouse secondary antibody and the avidin-biotin peroxidase complex, both supplied in the Vector Elite kit (Vector Laboratories, Burlingame, CA). Color was developed using the peroxidase substrate 4-chloro-1-naphthol/3,3-diaminobenzidine (Sigma). Sections were imaged via fluorescence microscopy (Nikon, Japan) using a confocal microscope (Bio-Rad). Membrane and pericyte immunostaining were performed using antibodies against p50 and p65 to determine the localization of the NF-κB complex.

Cell death in vitro. Cell death in vitro was assessed by three different methods.

Trypsin blue exclusion assay. BRECs were incubated for 5 days in 5 or 25 mM d-glucose, with or without different concentrations of PJ-34 or SN-50. Cell death was determined by the trypan blue exclusion assay. Briefly, an aliquot of the cell suspension was diluted 1:1 (vol/vol) with 0.1% trypan blue (Sigma), and the cells were counted with a hemocytometer. Cell death was defined as the percentage of blue-stained cells (dead cells) versus the total number of cells. Approximately 200–400 cells were counted in each sample, and each treatment was done in triplicate. The experiment was repeated three times.

Annexin V and propidium iodide staining. BRECs were incubated with 5 or 25 mM d-glucose with or without PJ-34 for 3 days and then cultured on chambered covers glasses (Fisher, Pittsburgh, PA) for 2 more days. Annexin V and PI staining was used to determine the cell death using a commercial available kit (Annexin-V-FLUOS staining kit; Roche). Cell death detection enzyme-linked immunosorbent assay. After 4 days in 5 or 25 mM d-glucose, with or without PJ-34, BRECs were collected using Trypsin-EDTA (0.5 mg/ml) and the nuclei were washed with PBS. Apoptotic cell death was determined by using a commercial available kit (Cell death detection ELISA kit; Roche) reported to be selective for apoptosis.

Quantitative measurement of leukostasis. Blood was removed from the vasculature of anesthetized animals (100 mg/kg Ketaset/100 mg/kg Xylazine = 5:1) by complete perfusion with PBS via a heart catheter. Animals were then perfused with fluorescein-coupled cancanavalin A lectin (20 μg/ml in PBS) through the aorta. Flat-mounted retinas (poly(ADP-ribose) groups) were incubated in a 1:100 dilution (Alexis) as previously described (24,25). Flat-mounted retinas were then counterstained with a monoclonal antibody against factor VIII (Alexis). The number of leukocytes adhering to the retinal vasculature was determined at 12 weeks of diabetes. The number of leukocytes adhering to the retinal vasculature was determined at 12 weeks of diabetes. The number of leukocytes adhering to the retinal vasculature was determined at 12 weeks of diabetes.
groups had significantly higher values than the corresponding ones in the nondiabetic control rats (Table 1). Diabetic rats were treated with insulin so that they did not lose weight, but failed to gain weight compared with nondiabetic rats. Body weights of both groups of diabetic rats remained significantly lower than those of nondiabetic control rats. Long-term administration of PJ-34 did not adversely affect the health or lifespan of diabetic rats.

**Diabetes induces PARP activation in retina and retinal capillary cells.** PARP activity was demonstrated using a monoclonal antibody to detect poly(ADP-ribosyl)ated proteins, the product of the enzyme. As shown in Fig. L4, there was marked increase in poly(ADP-ribosylation)ation of proteins from the retinal extract of 12-week diabetic rats compared with nondiabetic controls, and this was significantly inhibited by PJ-34. Immunostaining to detect sites of PARP activity revealed that PARP activity was increased slightly in nuclei of the ganglion cell layer, inner nuclear layer, and outer nuclear layer of diabetic rats (Fig. 1B). PJ-34 inhibited PARP activation in each of these sites. Because diabetic retinopathy is a vascular disease, special effort was directed to assessing PARP activity within the retinal vasculature. Little or no PARP activity was detected in freshly isolated retinal vasculature from nondiabetic animals, whereas PARP activity was demonstrated in about one-half of all capillary endothelial cells and pericytes in the freshly isolated retinal vasculature from diabetic animals. Thus, PARP was activated in nonvascular as well as microvascular cells of the retina in diabetes.

**Inhibition of PARP prevents the diabetes-induced death of retinal microvascular cells and early lesions of diabetic retinopathy.** Accelerated death of capillary cells is believed to be the major cause of acellular capillary formation (26). We used the TUNEL assay to assess DNA damage and apoptosis of cells in trypsin-digested retinal vasculature preparations (Fig. 2A). There was a threefold increase in the number of TUNEL-positive capillary cells (endothelial cells and pericytes) in the retinas of 36-week diabetic animals compared with nondiabetic controls (P < 0.0001), and administration of PJ-34 prevented this increase in cell death (P < 0.0001 compared with diabetic controls).

The number of acellular capillaries was significantly increased in retinas from rats diabetic for 36 weeks compared with age-matched control rats (1.8-fold of control, P < 0.0001), and this increase was significantly inhibited by PJ-34 (P < 0.0001) (Fig. 2B). The number of pericyte ghosts was also significantly increased in retinal vessels from diabetic rats compared with age-matched control rats (2.4-fold of control, P < 0.0005), and this increase also was significantly inhibited by PJ-34 (P < 0.02) (Fig. 2C).

The ability of PJ-34 to inhibit retinal endothelial cell death was also studied in vitro. By three different methods to assess cell death (trypan blue exclusion, annexin V staining, and cell death detection ELISA), 25 mmol/l glucose significantly increased BREC death compared with 5 mmol/l glucose (Fig. 3A–C). All of the methods showed that PJ-34 significantly inhibited the hyperglycemia-induced increase in endothelial death.

**TABLE 1**

Glycemia and body weights in the experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Duration (weeks)</th>
<th>Body weight (g)</th>
<th>Nonfasting blood glucose (mg/dl)</th>
<th>GHb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic</td>
<td>10</td>
<td>12</td>
<td>405 ± 27</td>
<td>62 ± 9</td>
<td>4.9 ± 1.0</td>
</tr>
<tr>
<td>Diabetic</td>
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<td>12</td>
<td>263 ± 19</td>
<td>327 ± 32</td>
<td>10.7 ± 2.8</td>
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<tr>
<td>Diabetic + PJ-34</td>
<td>7</td>
<td>12</td>
<td>268 ± 20</td>
<td>288 ± 43</td>
<td>12.5 ± 1.1</td>
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<td>Nondiabetic</td>
<td>28</td>
<td>36</td>
<td>575 ± 51</td>
<td>88 ± 26</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>19</td>
<td>36</td>
<td>314 ± 45</td>
<td>340 ± 81</td>
<td>10.2 ± 1.4</td>
</tr>
<tr>
<td>Diabetic + PJ-34</td>
<td>19</td>
<td>36</td>
<td>268 ± 31</td>
<td>372 ± 78</td>
<td>9.9 ± 1.0</td>
</tr>
</tbody>
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Data are means ± SD.

**FIG. 1.** PARP activation in diabetic retinas. A: Poly(ADP-ribosylation)ation was increased in retinas from rats diabetic for 12 weeks, and PJ-34 blocked this protein modification. N, nondiabetic rats; D, diabetic rats; D+PJ-34, diabetic rats treated with PJ-34. β-Actin is shown as a protein-loading control. B: PJ-34 blocked increased PARP activation in the ganglion cell layer (GCL), inner nuclear layer (INL), and outer nuclear layer (ONL) of diabetic rats (D). Poly(ADP-ribosyl)ated proteins stained gray and nuclei stained red. N, nondiabetic rats; D+PJ-34, diabetic rats treated with PJ-34. C: PARP activation was evaluated in retinal blood vessels isolated from nondiabetic (N) and diabetic (D) rats. Conventional stained blood vessels are shown on the left. An immunostained pericyte is illustrated by the red arrowhead, and a representative stained endothelial cell is indicated by the black arrow. H&E, hematoxylin and eosin.
PARP directly binds to NF-κB in cultured retinal cells. To further examine how PARP influences death of retinal capillary cells in elevated glucose, we initially focused on its ability to regulate the transcription factor, NF-κB. To test whether PARP was able to physically associate with NF-κB, we performed coimmunoprecipitation followed by Western blot analysis. Nuclear extracts of BRECs were immunoprecipitated with either an anti-p50 (Fig. 4A) or anti-p65 (Fig. 4B) antibody in 5 and 25 mmol/l glucose, and bound proteins were subsequently probed by Western blot using specific antibody against PARP. PARP was detected when immunoprecipitated with antibody against either p65 or p50, indicating that endogenous PARP and NF-κB form a complex in nuclei. More PARP was complexed to p50 at 25 mmol/l glucose than at 5 mmol/l glucose (Fig. 4A). In contrast, high glucose may have slightly decreased the association of PARP with the p65 subunit (Fig. 4B).

Inhibition of PARP inhibits the hyperglycemia-induced activation of NF-κB in retinal endothelial cells. Because PARP binds to NF-κB, we explored whether the enzyme activity of PARP was required for regulating NF-κB binding activity in retinal endothelial cells. Nuclear DNA binding activity of NF-κB in BRECs was examined by EMSA. Supershift assay demonstrated a classical p50/p65 heterodimer in this cell type (data not shown). There was increased DNA binding activity of NF-κB in cells incubated with 25 mmol/l glucose compared with that in 5 mmol/l glucose (Fig. 4C), and PJ-34 significantly inhibited this activation. The data suggest that poly(ADP-ribose)ylation influences the interaction between NF-κB and DNA. Inhibition of glucose-induced death in vitro by inhibition of NF-κB. Because PARP binds to NF-κB and regulates its activation, we evaluated whether PARP activation regulates the glucose-induced cell death via NF-κB activation. SN-50 is a cell-permeable peptide that binds to the nuclear translocation signal sequence of the p50 subunit of NF-κB, thus preventing NF-κB translocation to the nucleus. After 5 days’ incubation in 5 or 25 mmol/l glucose with or without 6 μmol/l SN-50, the trypan blue exclusion assay was used to quantitate endothelial cell death. Death of BRECs incubated in 25 mmol/l glucose was significantly greater than that in 5 mmol/l glucose (P < 0.05), and this hyperglycemia-induced increase in cell death was significantly inhibited by SN-50 (Fig. 5) (P < 0.05), suggesting that endothelial cell death in elevated glucose is at least partially mediated via NF-κB activation. NF-κB is activated in retinal endothelial cells from diabetic animals. To address whether p50 is increased in nuclei of retina endothelial cells in diabetes, we did p50 immunostaining on the trypsin-digested retinal vasculature in diabetic and nondiabetic animals. There was posi-
Diabetes-induced induction of ICAM-1 and leukostasis are mediated via PARP activation. ICAM-1 expression is known to be regulated by NF-κB and plays a critical role in the diabetes-induced adherence of leukocytes to the vessel wall (27). Diabetes of 12 weeks' duration increased ICAM-1 expression 2.5-fold in the retinal extract of diabetic rats compared with nondiabetic controls (Fig. 7A) (P < 0.05). Chronic inhibition of PARP by PJ-34 significantly inhibited the upregulation of ICAM-1 in diabetic retina (P < 0.05), suggesting that PARP regulates ICAM-1 expression via regulation of NF-κB.

Leukostasis in the retinal microcirculation was significantly greater than normal in the arteriolar, venular, and microvascular portions of the retinal vasculature of 12-week diabetic rats (P < 0.005, P < 0.001, and P < 0.001, respectively) (Fig. 7B). Treatment of the diabetic animals with PJ-34 significantly inhibited the diabetes-induced increase in leukostasis in the retinal vasculature compared with control diabetic rats (P < 0.005, P < 0.05, and P < 0.005, respectively).

**DISCUSSION**

An early and important lesion in the development of diabetic retinopathy is death of capillary cells (26), resulting in an increased number of acellular capillaries and pericyte ghosts. Acellular capillaries are of interest because they are not perfused (28) and thus are causally related to the development of retinal ischemia and subsequent neovascularization. In the present study, we found that PARP activity regulated the diabetes-induced death of retinal capillary cells. PARP inhibition prevented both the hyperglycemia-induced death of retinal endothelial cells and the diabetes-induced increase in the number of acellular capillaries and pericyte ghosts in retina. Methods used for the in vivo (TUNEL) and in vitro (annexin V staining and ELISA) studies suggest that many of the cells dying in an elevated glucose concentration are doing so by an apoptosis-like process.

PARP inhibition or PARP deficiency is known to downregulate various mechanisms of cell death, including mitochondrial permeability transition, mitochondrial oxidant generation, and the release of the cell death mediator apoptosis-inducing factor (29,30). Recently, it was reported that PARP regulated several genes of apoptotic regulators, including caspase-1 and -3 (31). Which apoptotic pathways are activated in diabetic retinopathy and how PARP regulates them needs to be further investigated.

The mechanism by which PARP causes endothelial cell dysfunction could be via transcriptional regulation. In other cells (8,32,33) and now in retinal endothelial cells, PARP has been found to directly bind to subunits of NF-κB and to regulate its transcriptional activity. In vitro, we showed that diabetic-like concentrations of glucose resulted in more binding of PARP to the p50 subunit of NF-κB compared with that in normal glucose levels. We also showed that inhibition of PARP activity significantly inhibited the hyperglycemia-induced activation of NF-κB by EMSA. Our data suggest that increased DNA binding affinity of NF-κB in retinal endothelial cells exposed to elevated glucose is mediated via PARP activity, which is consistent with a prior report that the binding of p50 to DNA is dependent on poly(ADP-ribosyl)ation (33). Interestingly, others (34) recently found that Drosophila mutants lacking normal PARP levels display immune defects similar to those in mice lacking the p50 subunit. Increased interaction between p50 and PARP in elevated glucose may help assemble other coactivators of NF-κB, such as histone acetyltransferase p300 (35,36), to form a transcriptional complex and regulate NF-κB–dependent gene expression.

Retinal NF-κB is activated early in diabetes and remains activated for up to 14 months (24,37). NF-κB is apparently
activated also in the retinal vasculature in diabetes, inasmuch as there is increased accumulation of the p50 subunit of NF-κB in nuclei of retinal endothelial cells of diabetic animals. In contrast to findings with the p50 subunit, a prior report (20) demonstrated that retinal microvascular cells from diabetic humans and rats had increased p65 expression in nuclei of pericytes but not endothelial cells. More study will be necessary to understand the significance of this diabetes-induced difference in the binding of NF-κB subunits to DNA. Our in vitro studies demonstrated that hyperglycemia caused an increase in DNA binding activity of NF-κB in retinal endothelial cells, in contrast to an absence of increasing DNA binding activity of the same cell type in the prior study. The basis of this difference is not known, but data from this study and publications by Adamis et al. (27) showed that ICAM-1 (an endothelial protein regulated by NF-κB) is increased in retinas of diabetic animals, consistent with our data that NF-κB activity is increased in retinal endothelial cells in hyperglycemia.

The relative contribution of NF-κB transcriptional activity to either prosurvival or proapoptotic pathways depends on multiple factors, including intensity of the activating stimulus, cell type, and activation of other transcription factors (38–40). We report here that inhibition of NF-κB translocation to the nucleus with SN-50 inhibited death of retinal endothelial cells induced by an elevated glucose concentration in vitro, suggesting that NF-κB activation has at least some adverse effects in the retina in diabetes. These findings further support developing evidence (22,24,25,27,41–45) that inflammatory processes strongly contribute to the pathogenesis of diabetic retinopathy. Many effects of NF-κB activation are mediated via its regulation of inflammatory processes (40,46), and PARP also has an important role in inflammatory disorders as a coactivator of NF-κB (9).

PARP-induced activation of NF-κB likely results in at least two mechanisms that might contribute to capillary cell death in diabetes. In vivo, increased NF-κB–mediated transcription leads to increased expression of ICAM-1 on endothelial cells, resulting in excessive leukostasis in retinal vessels and possibly vaso-occlusion. Additionally, our finding that hyperglycemia-induced cell death was also regulated in vitro by PARP and NF-κB demonstrates that there is also another NF-κB–mediated route to cell death that does not require leukostasis or vaso-occlusion. PARP activation might also cause cell death independent of NF-κB activation. The best recognized of these alternate mechanisms is the depletion of NAD+/ATP by PARP overactivation (3). Recently, PARP has also been shown (47) to

FIG. 6. Increased p50 expression in nuclei of endothelial cells. A: p50 immunostaining in nuclei from the retinal vasculature of a 12-week diabetic rat is shown on the left, and the lack of positive immunostaining following preincubation of the p50 antibody with p50 blocking peptide is shown on the right. Positive p50 immunostaining appears dark gray, and the counterstained nuclei appear light gray. B: p50-positive stained nuclei of endothelial cells were counted as described in the RESEARCH DESIGN AND METHODS and reported as stained endothelial nuclei per 100 capillary cells. *P < 0.02 vs. nondiabetic control. D, diabetic rat; N, nondiabetic rat.

FIG. 7. PARP inhibitor prevents hyperglycemia-induced ICAM-1 expression and subsequent leukostasis. A: ICAM-1 protein expression was measured in retinal homogenates of nondiabetic rats (N; n = 3), diabetic rats (D; n = 3), and diabetic rats treated with PJ-34 (D+PJ-34; n = 3). Expression is normalized to nondiabetic control, set as 100%. *P < 0.05 vs. nondiabetic control; **P < 0.05 vs. diabetic control rats. B: Inhibition of the diabetes-induced increase in leukostasis in animals fed the PARP inhibitor for 12 weeks. Leukocyte adhesion was quantified in arteriolar, venular, and microvascular portions of the retinal vasculature. N, nondiabetic rats (n = 10); D, diabetic rats (n = 10); D+PJ-34, diabetic rats treated with PJ-34 (n = 7). *P < 0.005 vs. nondiabetic control; **P < 0.05 vs. diabetic rats.
mediate a partial inhibition of the glycolytic pathway in elevated concentrations of glucose, thus causing several metabolic abnormalities (including activation of NF-κB) that have been postulated to contribute to the development of diabetic complications.

Recent work has demonstrated the importance of PARP activation in diabetes-induced alterations in the function of macrovascular endothelium, cardiomyocytes, and neurons (48–50). Here we demonstrate that PARP activation also mediates the hyperglycemia-induced death of retinal capillary endothelial cells and development of at least the early stages of diabetic retinopathy. Our study suggests the special importance of the PARP-mediated activation of NF-κB in the pathogenesis of the retinopathy and thus offers several novel targets at which development of diabetic retinopathy might be inhibited.

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