Published in final edited form as:

Free Radic Biol Med. 2011 May 15; 50(10): 1400–1409. doi:10.1016/j.freeradbiomed.2011.01.037.

PARP INHIBITION ALLEVIATES DIABETES-INDUCED SYSTEMIC OXIDATIVE STRESS AND NEURAL TISSUE 4-HYDROXYNONENAL ADDUCT ACCUMULATION: CORRELATION WITH PERIPHERAL NERVE FUNCTION

Sergey Lupachyk, Hanna Shevalye, Yury Maksimchyk, Viktor R. Drel, and Irina G. Obrosova

Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, LA

Abstract

This study evaluated the role of poly(ADP-ribose) polymerase in systemic oxidative stress and 4-hydroxynonenal adduct accumulation in diabetic peripheral neuropathy. Control and streptozotocin-diabetic rats were maintained with or without treatment with the PARP inhibitor, 1,5-isoquinolinediol, 3 mg kg⁻¹d⁻¹, for 10 weeks after initial 2 weeks. Treatment efficacy was evaluated by poly(ADP-ribosyl)ated protein content in peripheral nerve and spinal cord (Western blot analysis) and dorsal root ganglion neurons and non-neuronal cells (fluorescent immunohistochemistry), as well as by indices of peripheral nerve function. Diabetic rats displayed increased urinary isoprostane and 8-hydroxy-2'-deoxyguanosine excretion (ELISA), 4-hydroxynonenal adduct accumulation in endothelial and Schwann cells of the peripheral nerve, neurons, astrocytes, and oligodendrocytes of the spinal cord, and neurons and glial cells of the dorsal root ganglia (double-label fluorescent immunohistochemistry) as well as motor and sensory nerve conduction velocity deficits, thermal hypoalgesia, and tactile allodynia. PARP inhibition counteracted diabetes-induced systemic oxidative stress and 4-hydroxynonenal adduct accumulation in peripheral nerve and spinal cord (Western blot analysis) and dorsal root ganglion neurons (perikarya, fluorescent immunohistochemistry) which correlated with improvement of large and small nerve fiber function. The findings reveal the important role of PARP activation in systemic oxidative stress and 4-hydroxynonenal adduct accumulation in diabetic peripheral neuropathy.

Keywords

4-hydroxynonenal adduct; 1,5-isoquinolinediol; peripheral diabetic neuropathy; poly(ADP-ribose) polymerase; systemic oxidative stress

Diabetic peripheral neuropathy (DPN) affects at least 50% of diabetic subjects with both Type 1 and Type 2 diabetes mellitus in the United States, and is the leading cause of foot amputation [1–4]. Pathogenetic mechanisms of DPN include, but are not limited to, activation of aldose reductase [5–8], advanced glycation end-product/receptor for advanced

Address for correspondence and reprint request: Irina G.Obrosova, Ph.D., Pennington Biomedical Research Center, Louisiana State University, 6400 Perkins Road, Baton Rouge, LA 70808, Tel. (225) 763-0276, FAX. (225) 763-0274, obrosoig@pbrc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

glycation end-product axis [8,9], protein kinase C [6,11], and mitogen-activated protein kinases [12,13], changes in arachidonic acid metabolism [14,15], and impaired neurotrophic support [16–18]. Many of these mechanisms have been identified in both vascular endothelium and neural elements [dorsal root ganglion (DRG) neurons, Schwann cells] of the peripheral nerve as well as in the spinal cord.

Growing evidence suggests the important contribution of oxidative stress and poly(ADP-ribose) polymerase (PARP) activation to endoneurial nutritive blood flow and motor and sensory nerve conduction velocity (MNCV and SNCV) deficits, sensory neuropathy, as well as large and small sensory nerve fiber degeneration [19–25]. Both phenomena have been documented in the peripheral nervous system of animal models of Type 1 and Type 2 diabetes as well as prediabetes (reviewed in [26]). In the best-studied animal model for DPN i.e., streptozotocin (STZ)-diabetic rat, oxidative stress and poly(ADP-ribosyl)ation have been identified in neurons and Schwann cells of the peripheral nerve and in *vasa nervorum* [21,22,25,27–29]. Furthermore, both oxidative stress and PARP activation were clearly manifest in microvasculature of human subjects with diabetes mellitus [30]. For many years, free radical and oxidant-induced DNA single-strand breakage was considered an obligatory step for PARP activation [31]. However, recent studies including those related to diabetic complications suggest that 1) PARP is also activated by metabolic factors e.g., through phosphorylation by extracellular signal regulated kinase [32]; and 2) diabetes-induced poly(ADP-ribosyl)ation is clearly manifest in tissues in which DNA single-strand breakage is negligible or totally absent, e.g., Schwann cells of the peripheral nerve [22,33]. It has also been shown that PARP activation induced by both high glucose and non-esterified fatty acids, two major detrimental factors in the diabetic milieu, precedes and causes oxidative stress in a number of cell targets for diabetic complications including Schwann cells of the peripheral nerve [34], retinal pericytes and endothelial cells [35], and renal podocytes [36]. The afore-mentioned studies have predominantly been performed in *in vitro* cultured cells, and used cell-permeable agents generating fluorescent compounds in reactions with intracellular reactive oxygen species (2',7'-dichlorodihydrofluorescein diacetate, hydroethidine) for oxidative stress evaluation. Note, that the inter-experiment reproducibility of such measurements is quite low.

To further explore the relations between oxidative stress and PARP activation in diabetes, the present *in vivo* study evaluated the effect of the potent and specific inhibitor PARP inhibitor 1,5-isoquinolinediol on systemic oxidative stress and 4-hydroxynonenal protein adduct accumulation in tissue-targets for DPN i.e., peripheral nerve, spinal cord, and DRG neurons. We employed a well-studied STZ-diabetic rat model, which displays clearly manifest systemic and neural oxidative stress [21,23,25,27–29,37–39], and reliable quantitative techniques for evaluation of oxidative injury.

METHODS

A. Reagents

Unless otherwise stated, all chemicals were of reagent-grade quality, and were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO. Mouse monoclonal anti-poly(ADP-ribose) was purchased from Trevigen, Inc., Gaithersburg, MD, and rabbit polyclonal anti-4-hydroxynonenal-Michael adduct (4-HNE-adduct) antibody purchased from Calbiochem, San Diego, CA, mouse monoclonal anti-S-100 antibody was purchased from Santa Cruz, Santa Cruz, CA. Mouse monoclonal anti-glutamine synthetase, anti-NeuN, and anti-glial fibrillary acidic protein antibodies were obtained from Chemicon, Billerica, MA, and mouse monoclonal anti-APC-Ab7 from Calbiochem, San Diego, CA. Isolectin GS-IB4 from Griffonia simplicifolia, Alexa Fluor(R) 594 conjugate, goat anti-rabbit Alexa Fluor 488, goat anti-mouse Alexa Fluor 594, and Prolong Gold Antifade Reagent were purchased from

Molecular Probes, Eugene, OR. Avidin/Biotin Blocking Kit, VECTASTAIN Elite ABC Kit (Standard*), DAB Substrate Kit, and 3,3'-diaminobenzidine were obtained from Vector Laboratories, Burlingame, CA. Other reagents for immunohistochemistry have been purchased from Dako Laboratories, Inc., Santa Barbara, CA.

B. Animals

The experiments were performed in accordance with regulations specified by the National Institutes of Health "Principles of Laboratory Animal Care, 1985 Revised Version" and Pennington Biomedical Research Center Protocol for Animal Studies. Male Wistar rats (Charles River, Wilmington, MA), body weight 250–300 g, were fed a standard rat chow (PMI Nutrition Int., Brentwood, MO) and had access to water *ad libitum*. Streptozotocin (STZ)-diabetes was induced as described [22]. Blood samples for glucose measurements were taken from the tail vein ~48 h after the STZ injection and the day prior to the study termination. All rats with blood glucose levels ≥ 13.8 mM were considered diabetic. Diabetic rats were maintained on suboptimal doses of insulin (~1–2 U every second day) to prevent ketoacidosis and weight loss. The experimental groups comprised control and diabetic rats treated with or without the PARP inhibitor 1,5-isoquinolinediol (ISO), 3 mg kg⁻¹d⁻¹ i.p., for 10 wks after first 2 wks without treatment. An initial 2-wk period without treatment was introduced to avoid β -cell regeneration and alleviation of hyperglycemia which is known to occur when PARP inhibitors are administered together with streptozotocin or shortly after induction of diabetes. At the end of the study, rats were placed in individual metabolic cages (Lab Products Inc., Seaford, Delaware) and urine collected for 24 h. Urine specimens were centrifuged at 12,000 g (4°C, 10 min) and frozen for subsequent assessment of 8-isoprostane and 8-hydroxy-2'-deoxyguanosine by ELISA.

C. Anesthesia, euthanasia and tissue sampling

The animals were sedated by CO₂, and immediately sacrificed by cervical dislocation. Sciatic nerves and spinal cords were rapidly frozen in liquid nitrogen, and stored at -80°C prior to assessment of poly(ADP-ribosyl)ated proteins and 4-HNE-adducts by Western blot analyses. A portion of sciatic nerves and spinal cords as well as DRG from untreated diabetic group were fixed in 4% formalin and later used for 4-HNE-adduct localization to individual cell types in peripheral nervous system (PNS), using double-label fluorescent immunohistochemistry with specific cell markers, as well as extranuclear and intranuclear poly(ADP-ribose) localization (sciatic nerve only). The rest of 4% formalin-fixed DRG was used for assessment of 4-HNE-adduct and poly(ADP-ribose) abundance by fluorescent immunohistochemistry.

D. Specific methods

D.1. Motor and sensory nerve conduction velocities—Sciatic motor and hind-limb digital sensory nerve conduction velocities (MNCV and SNCV) were assessed at three time points i.e., at the beginning of the study (prior to induction of diabetes), at 2 wks (prior to interventions), and at 12 wks (final measurements), as we described previously [22].

D.2. Behavioral tests—Assessment of thermal allodynia (by paw withdrawal latencies), mechanical allodynia (rigid von Frey filament test), and tactile allodynia (flexible von Frey filament test) was performed at three time points i.e., at the beginning of the study (prior to induction of diabetes), at 2 wks (prior to interventions), and at 12 wks (final measurements), as we described previously [22–24].

D.3. Western blot analysis of 4-HNE-adducts and poly(ADP-ribosyl)ated proteins in sciatic nerve and spinal cord—Western blot analysis of 4-HNE-adducts

and poly(ADP-ribosyl)ated proteins in sciatic nerve and spinal cord was performed, as we described in detail previously [15,25]. We employed 5–17% sodium dodecyl sulfate-polyacrylamide gels, and the electro-phoresis was conducted for 2 h. Protein bands were visualized with the BM Chemiluminescence Blotting Substrate (POD) (Roche, Indianapolis, IN). Membranes were then stripped and reprobed with β -actin antibody to verify equal protein loading. The data were quantified by densitometry (Quantity One 4.5.0 software, Bio-Rad Laboratories, Richmond, CA).

D.4. Fluorescent immunohistochemistry of 4-HNE-adducts and poly (ADP-ribose) in DRG—All sections were processed by a single investigator and evaluated blindly. 4-HNE-adduct and poly(ADP-ribose) abundance was assessed by fluorescent immunohistochemistry as described [25]. Low power observations of DRG sections stained for 4-HNE-adducts and poly(ADP-ribose) were made using a Zeiss Axioplan 2 imaging microscope. Color images were captured with a Photometric CoolSNAPTTM HQ CCD camera at 1392×1040 resolution. Low power images were generated with a 40X acroplan objective using the RS ImageTM 1.9.2 software. 4-HNE-adduct and poly(ADP-ribose) fluorescence intensity of individual DRG neurons was quantified using the ImageJ 1.43q software (National Institutes of Health, Bethesda, MD), and normalized per neuronal area. Because of highly variable size of individual nuclei, and low nuclear HNE-adduct fluorescence, the latter was subtracted from total neuronal HNE-adduct fluorescence. 43–110 and 53–177 neurons per experimental group were counted for assessment of 4-HNE-adduct and poly(ADP-ribose) fluorescence, respectively. Poly(ADP-ribose) fluorescence intensity of individual non-neuronal cells was quantified with the program described above and normalized per satellite cell area. 45–105 non-neuronal cells per experimental group were counted.

D.5. 4-HNE-adduct localization by double-label fluorescent immunohistochemistry—4-HNE is a highly reactive α,β -unsaturated aldehyde formed from the breakdown of polyunsaturated fatty acids in cholesterol esters, phospholipids and triglycerides, and is a major product of lipid peroxidation. It is known to inhibit protein and DNA synthesis, inactivate enzymes, form protein adducts with amino acid residues such as cysteine, histamine and lysine, and act as a signaling molecule (reviewed in [40]). In the peripheral nervous system, 4-HNE inhibits neurite outgrowth [41]. Modification of proteins by 4-HNE has been proposed to cause neurotoxicity in a number of neurodegenerative diseases, including distal axonopathy in diabetic sensory neuropathy [26,40,42]. For assessment of 4-HNE protein adducts in the present study, the sections of sciatic nerve, spinal cord, and DRG were deparaffinized in xylene, hydrated in decreasing concentrations of ethanol, and washed in water. For double fluorescent immunohistochemistry of the sciatic nerve, anti-4-HNE antibody (1:500 dilution) was applied in combination with isolectin Alexa Fluor 594 conjugate or anti-S-100 antibody (1:100 dilution), for localization of 4-HNE to endothelial and Schwann cells, respectively. To localize 4-HNE to neurons, oligodendrocytes, and astrocytes of the spinal cord, anti-4-HNE antibody (1:500 dilution) was applied in combination with anti-NeuN, anti-APC-Ab7, or anti-GFAP antibodies diluted 500, 100, and 200 times, respectively. A combination of anti-4-HNE and anti-glutamine synthetase antibodies (diluted 500 and 200 times, respectively) was used to localize 4-HNE to glial cells of DRG. After incubation with primary antibodies, all the sections were washed in PBS containing 0.01% Triton X-100. A cocktail of secondary Alexa Fluor 488 and 594 antibodies (both in 1:150 dilution) or secondary Alexa Fluor 488 antibody alone (1:150 dilution, for isolectin-stained sections only) were applied for 2 h at room temperature. Then the sections were washed, coverslipped, and mounted with Kaiser's glycerin-gelatin (Merck, Darmstadt, Germany). The Adobe Photoshop 7.0 program (Adobe

Systems Inc., San Jose, CA) was used to merge the images. To verify specificity of all immunostainings, primary antibodies were omitted in negative controls.

D.6. Extranuclear and intranuclear localization of poly(ADP-ribose) in sciatic nerve—The sections of sciatic nerve were processed as described above. Anti-poly(ADP-ribose) antibody (1:100 dilution) was applied in combination with 4',6-diamidino-2-phenylindole, a fluorescent stain that forms fluorescent complexes with natural double-stranded DNA. After incubation with primary antibody, all the sections were washed in PBS containing 0.01% Triton X-100. A secondary Alexa Fluor 594 antibody (in 1:150 dilution) or no antibody (for DAPI-stained sections only) was applied for 2 h at room temperature. Then the sections were washed, coverslipped, and mounted with Kaiser's glycerin-gelatin (Merck, Darmstadt, Germany). The Adobe Photoshop 7.0 program (Adobe Systems Inc., San Jose, CA) was used to merge the images. To verify specificity of immunostainings, primary antibody was omitted in negative controls.

D.7. Urinary isoprostane and 8-hydroxy-2'-deoxyguanosine excretion—Urinary isoprostane, and 8-hydroxy-2'-deoxyguanosine excretions were assessed by ELISA. Urinary Isoprostane ELISA kit (Oxford Biomedical Research, Oxford, MI) and 8-hydroxy-2'-deoxy Guanosine EIA Kit (Cayman Chemical Company, Ann Arbor, MI) were used, and the assays were performed in accordance with the manufacturers' instructions.

E. Statistical analysis

The results are expressed as Mean $\pm$ SEM. Data were subjected to equality of variance F test, and then to log transformation, if necessary, before one-way analysis of variance. Where overall significance ($p < 0.05$) was attained, individual between group comparisons were made using the Student-Newman-Keuls multiple range test. Significance was defined at $p \leq 0.05$. When between-group variance differences could not be normalized by log transformation (datasets for body weights and plasma glucose), the data were analyzed by the nonparametric Kruskal-Wallis one-way analysis of variance, followed by the Bonferroni/Dunn or Fisher's PLSD tests for multiple comparisons.

RESULTS

The initial (prior to STZ administration) body weights were similar in control and diabetic rats treated with or without ISO (Table 1). The final body weights were similarly reduced in untreated and PARP inhibitor-treated diabetic rats compared with the control group. Initial blood glucose concentrations were increased 4.1-fold and 4.2-fold in untreated and ISO-treated diabetic rats, compared with non-diabetic controls. In a similar fashion, final blood glucose concentrations were 3.8- and 4-fold higher in untreated and ISO-treated diabetic rats than in non-diabetic controls. PARP inhibition did not affect either weight gain or blood glucose concentrations in non-diabetic rats.

Poly(ADP-ribose) fluorescence was localized within both intranuclear and extranuclear space of rats with 12-wk duration of STZ-diabetes (Fig. 1). Poly(ADP-ribosyl)ated protein levels were increased by 18% and 41% in the peripheral nerve and spinal cord of diabetic rats compared with non-diabetic controls (Fig. 2, A and B). Diabetes-associated increase in poly(ADP-ribosyl)ation in both tissues was essentially prevented by ISO. In both non-diabetic and diabetic rats, poly(ADP-ribose) fluorescence was more than 2-fold greater in DRG non-neuronal cells, than in DRG neurons. The diabetic condition increased poly(ADP-ribosyl)ation by 55% and 35% in DRG neurons and non-neuronal DRG cells, respectively (Fig. 3, A and B). ISO, 3 mg kg⁻¹d⁻¹, reduced poly(ADP-ribose) fluorescence by 33% in neurons and by 13% in non-neuronal cells of diabetic rats, without affecting this variable in

either cell type in non-diabetic controls. Apparently, the dose of ISO was not sufficient to completely inhibit higher poly(ADP-ribosyl)ation in non-neuronal cells. The difference in pharmacological disposition of ISO between DRG neurons and non-neuronal cells can also account for the observed differences in the extent of PARP inhibition.

MNCV and SNCV at three time points i.e., at the beginning of the study (prior to induction of diabetes), at the 2-week time point (prior to a pharmacological intervention), and at the end of the study, are presented in Table 2. Rats with both 2-wk and 12-wk (untreated group) durations of diabetes displayed MNCV and SNCV deficits. ISO treatment blunted motor and sensory nerve conduction slowing in diabetic rats without affecting either MNCV or SNCV in control rats. Thermal response latencies were reduced in rats with 2-wk and increased in rats with 12-wk diabetes, consistent with the development of early hyperalgesia followed by a loss of sensitivity to thermal noxious stimuli. ISO alleviated thermal hypoalgesia in diabetic rats, without affecting thermal response latencies in control rats. Mechanical withdrawal thresholds were reduced in rats with both 2-wk and 12-wk durations of diabetes, indicative of the development of mechanical hyperalgesia. PARP inhibition alleviated, although did not completely reverse, mechanical hyperalgesia in diabetic rats, without affecting this variable in non-diabetic controls. Tactile response thresholds were indistinguishable between non-diabetic rats and rats with 2-wk duration of diabetes. Rats with 12-wk duration of diabetes displayed tactile allodynia, which was partially prevented by ISO treatment. A PARP inhibitor did not affect tactile response thresholds in non-diabetic rats.

Rats with 12-wk duration of STZ-diabetes displayed clearly manifest systemic oxidative stress, with 4.8-fold and 2.2-fold increases in urinary isoprostane and 8-hydroxy-2'-deoxyguanosine excretions, respectively (Fig.4, A and B). ISO treatment reduced 8-hydroxy-2'-deoxyguanosine excretion in diabetic rats ($p < 0.05$) whereas isoprostane excretion tended to decrease, but the difference with the untreated diabetic group did not achieve statistical significance ($p = 0.25$).

4-HNE-adduct level was increased by 17% and 13% in the sciatic nerve and spinal cord of diabetic rats compared with controls ($p < 0.01$ for both), and this increase was essentially blunted by ISO treatment (Fig.5, A and B). 4-HNE-adduct fluorescence in DRG neurons (perikarya) was increased 4-fold in diabetic rats compared with controls (Fig.6). PARP inhibition reduced, but did not completely prevent, diabetes-associated neuronal 4-HNE-adduct accumulation. In diabetic rats, 4-HNE-adduct fluorescence was clearly identifiable in endothelial and Schwann cells of the peripheral nerve (Fig.7, A and B), neurons, oligodendrocytes, and astrocytes of the spinal cord (Fig.7, C–E), and neurons and glial cells of DRG (Fig.7, F).

DISCUSSION

The findings presented herein suggest the presence of clearly manifest systemic and neural oxidative stress and PARP activation in rats with a 12-wk duration of STZ-diabetes. Diabetes-induced 4-HNE adduct accumulation and poly(ADP-ribosyl)ation were revealed in peripheral nerve, spinal cord, and dorsal root ganglion neurons (perikarya) and satellite cells. PARP inhibition suppressed systemic oxidative stress and HNE adduct accumulation in tissue sites for PDN, which correlated with improvement of peripheral nerve function.

Systemic oxidative stress reflects an imbalance between ROS production and antioxidative defense in multiple organs and tissues. Systemic oxidative stress manifest, among other changes, by elevated serum malondialdehyde and nitrotyrosine concentrations, and increased urinary isoprostane and 8-hydroxy-2'-deoxyguanosine excretion, has been

described in diabetic animal models [37–39] as well as in human subjects with both Type 1 and Type 2 diabetes where it correlated with the presence and severity of diabetic complications [43–47]. Identification of systemic oxidative stress variables correlating with diabetic complications and amenable to pathogenetic treatments is very important considering infeasibility of evaluating oxidative injury in tissue-sites for DPN in humans. The findings of the present experimental study are in agreement with several clinical reports [43,44] suggesting that both urinary isoprostane and 8-hydroxy-2'-deoxyguanosine excretion should be studied further as potential biomarkers of the presence and severity of diabetic complications, and, in particular, PDN.

The finding of diabetes-associated peripheral nerve 4-HNE adduct accumulation is consistent with increased free malondialdehyde (MDA) plus 4-HNE concentration in our previous studies [48]. Note, however, that in contrast to free MDA plus 4-HNE [48], 4-HNE adduct level correlated with diabetes-induced peripheral nerve dysfunction. Importantly, 4-HNE adduct accumulation was identified in both endothelial and Schwann cells of diabetic peripheral nerve i.e., two cell types respectively responsible for decreased endoneurial nutritive blood flow and impaired neurotrophic support characteristic for PDN. Thus, our findings are consistent with the current knowledge on oxidative stress as a causative factor in both phenomena [19–21,49]. Identification of diabetes-associated 4-HNE adduct accumulation in neurons, astrocytes, and oligodendrocytes of the spinal cord is also important considering spinal involvement in altered electrophysiology and sensory neuropathy in human subjects with diabetes [50]. Note, that serum 4-HNE adduct concentration has recently been reported to correlate with the presence and severity of systemic lupus erythematosus and other autoimmune diseases [51]. It remains to be established whether 4-HNE adduct accumulation in blood serum, erythrocytes, or peripheral monocytes has diagnostic and prognostic value for DPN and other diabetic complications.

The presence of diabetes-associated oxidative-nitrosative stress manifest by increased 8-hydroxy-2'-deoxyguanosine and nitrotyrosine immunoreactivities in rat and mouse DRG neurons (perikarya) has previously been reported by several groups [23,27,29,52] including ours [23,52]. In contrast, others [53] found similar levels of oxidative stress (reactive oxygen species production assessed with two cell-permeable dyes) in DRG neurons (perikarya) from control rats and rats with 12-wk duration of STZ-diabetes. Note, however, that the aforementioned study has been performed in isolated and cultured DRG neurons which may or may not preserve their natural phenotype during isolation and cultivation. In addition, the authors did not perform cell counts and, the most important, did not normalize fluorescence of individual neurons to cell area (large and small neurons have several-fold differences in fluorescence signal). Taking into consideration the importance of exact identification of ROS targets in tissue-sites for diabetic complications for selection of valid biochemical endpoints for drug discovery and, especially, development of gene therapies [54,55], we performed assessment of 4-HNE-adduct fluorescence (immunohistochemistry) and normalized it to cell area in a large number of individual DRG neurons from control rats and rats with 12-wk duration of STZ-diabetes (i.e., the one used in the afore-mentioned study [53]). Our findings revealing diabetes-related four-fold increase in neuronal 4-HNE adduct fluorescence are consistent with demonstration of enhanced neuronal oxidative stress in DPN by non-quantitative immunohistochemistry by two other groups [27,29]. Furthermore, they are consistent with previous semiquantitative data from our group obtained in rats with shorter durations of STZ-diabetes [23], STZ-diabetic [52] and leptin-deficient (*ob/ob*) mice [56], and high-fat diet fed mice [34], as well as with the findings of high glucose-induced oxidative stress in adult sensory neurons obtained by others [57]. Note, that the significance of neuronal oxidative-nitrosative stress in the pathogenesis of PDN remains unclear, and two recent studies from our group suggest that oxidative stress in Schwann cells rather than DRG neurons is implicated in peripheral nerve dysfunction [52,58]. More focused approach,

potentially with gene therapies targeting oxidative stress selectively in neurons or Schwann cells, may provide new insights into the roles of oxidative stress in the two cell types in DPN.

PARP activation resulting in energy failure and inhibition of glucose oxidation, changes in transcriptional regulation and gene expression, signal transduction changes, and growth factor imbalances [31,59–62], plays a major role in diabetic complications including endothelial dysfunction [31,63], cardiomyopathy [64], retinopathy [35,65], nephropathy [36,61], as well as cataract [35]. The present study is first to demonstrate that PARP inhibition is associated with alleviation of diabetes-related systemic oxidative stress. It also confirms and expands earlier observations made by qualitative immunohistochemistry and by using cell-permeable dyes in cell culture models [36,66] suggesting that PARP activation contributes to oxidative-nitrosative stress in tissue and cell targets for diabetic complications, including DPN. Note, that ISO completely or essentially corrected diabetes-induced increase in 4-HNE adduct fluorescence in sciatic nerve, spinal cord, and DRG neurons, whereas the effect on systemic oxidative stress was modest. Apparently, PARP activation plays a more important role in oxidative stress in PNS than in other organs and tissues in diabetes. Theoretically, PARP activation can contribute to diabetes-induced oxidative stress via multiple mechanisms. First, PARP activation leads to depletion of the enzyme substrate, NAD⁺ [31], and, correspondingly, reduction in NAD(P)⁺/NAD(P)H ratios [67] which is likely to be associated with increased NAD(P)H oxidase activity [68,69]. Second, PARP activation is responsible for nuclear factor- κ B-mediated expression of pro-inflammatory cytokines [60], known to cause oxidative-nitrosative stress. Third, diabetes-induced PARP activation leads to the formation of methylglyoxal-derived advanced glycation end-products, known to generate oxidative stress via interaction with their receptors [61]. Finally, PARP has been implicated in phosphorylation of p38-mitogen-activated protein kinase [62], which participates in GSH depletion in diabetic peripheral nerve [70]. Similarly to our previous findings with several antioxidants and PARP inhibitors (reviewed in [26]), reduction of oxidative stress and PARP inhibition in peripheral nerve, spinal cord, and DRG correlated with improvement of peripheral nerve function, and, in particular, alleviation of MNCV and SNCV deficits, thermal hypoalgesia, mechanical hyperalgesia, and tactile allodynia. This is in line with observations of others implicating oxidative stress and PARP activation in the pathogenesis of DPN and other diabetic complications.

In conclusion, PARP inhibition reduced systemic oxidative stress and 4-HNE accumulation in sciatic nerve, spinal cord, and DRG neurons which correlated with alleviation of peripheral nerve dysfunction. The role for PARP activation in mechanisms of free radical production and antioxidative defense in tissue-sites for DPN requires further studies. Identification of the role for PARP activation in diabetes-related oxidative stress, consistent with previous reports on the important contribution of both mechanisms to the pathogenesis of diabetic complications [19–21,28,29,31,33,35,36,59,63–65], supports rationale for development and further studies of PARP inhibitors and PARP inhibitor-containing combination therapies. The link between PARP activation and oxidative stress should also be studied in other pathological conditions that involve both phenomena and, in particular, in cancer [31,59], especially with consideration of the ongoing trials identifying the PARP inhibitor olaparib as a potential treatment for different types of malignant tumors [71,72].

Abbreviations

DPN	diabetic peripheral neuropathy
DRG	dorsal root ganglion

4-HNE	4-hydroxynonenal
ISO	1,5-isoquinolinediol
MDA	malondialdehyde
MNCV	motor nerve conduction velocity
PARP	poly(ADP-ribose) polymerase
PBS	phosphate-buffered saline
PDN	peripheral diabetic neuropathy
PNS	peripheral nervous system
ROS	reactive oxygen species
SNCV	sensory nerve conduction velocity
STZ	streptozotocin

Acknowledgments

The study was supported by the National Institutes of Health Grants RO1DK074517, RO1DK077141, and RO1DK081147 (all to IGO). The Cell Biology and Bioimaging Core utilized in this work was supported in part by COBRE (NIH P20 RR021945) and CNRU (NIH 1P30-DK072476) center grants from the National Institutes of Health.

REFERENCES

1. Boulton AJ. The diabetic foot: from art to science. The 18th Camillo Golgi lecture. *Diabetologia*. 2004; 47:1343–1353. [PubMed: 15309286]
2. Boulton AJ, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, Malik RA, Maser RE, Sosenko JM, Ziegler D. Diabetic neuropathies: a statement by the American Diabetes Association. *Diabetes Care*. 2005; 28:956–962. [PubMed: 15793206]
3. Veves A, Backonja M, Malik RA. Painful diabetic neuropathy: epidemiology, natural history, early diagnosis, and treatment options. *Pain Med*. 2008; 9:660–674. [PubMed: 18828198]
4. Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L, Valensi P. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*. 2010; 33:2285–2293. [PubMed: 20876709]
5. Cameron NE, Cotter MA, Basso M, Hohman TC. Comparison of the effects of inhibitors of aldose reductase and sorbitol dehydrogenase on neurovascular function, nerve conduction and tissue polyol pathway metabolites in streptozotocin-diabetic rats. *Diabetologia*. 1997; 40:271–281. [PubMed: 9084964]
6. Nakamura J, Kato K, Hamada Y, Nakayama M, Chaya S, Nakashima E, Naruse K, Kasuya Y, Mizubayashi R, Miwa K, Yasuda Y, Kamiya H, Ienaga K, Sakakibara F, Koh N, Hotta N. A protein kinase C-beta-selective inhibitor ameliorates neural dysfunction in streptozotocin-induced diabetic rats. *Diabetes*. 1999; 48:2090–2095. [PubMed: 10512378]
7. Yagihashi S, Yamagishi SI, Wada RR, Baba M, Hohman TC, Yabe-Nishimura C, Kokai Y. Neuropathy in diabetic mice overexpressing human aldose reductase and effects of aldose reductase inhibitor. *Brain*. 2001; 124:2448–2458. [PubMed: 11701599]
8. Ho EC, Lam KS, Chen YS, Yip JC, Arvindakshan M, Yamagishi S, Yagihashi S, Oates PJ, Ellery CA, Chung SS, Chung SK. Aldose reductase-deficient mice are protected from delayed motor nerve conduction velocity, increased c-Jun NH2-terminal kinase activation, depletion of reduced glutathione, increased superoxide accumulation, and DNA damage. *Diabetes*. 2006; 55:1946–1953. [PubMed: 16804062]

9. Bierhaus A, Haslbeck KM, Humpert PM, Liliensiek B, Dehmer T, Morcos M, Sayed AA, Andrassy M, Schiekofer S, Schneider JG, Schulz JB, Heuss D, Neundorfer B, Dierl S, Huber J, Tritschler H, Schmidt AM, Schwaninger M, Haering HU, Schleicher E, Kasper M, Stern DM, Arnold B, Nawroth PP. Loss of pain perception in diabetes is dependent on a receptor of the immunoglobulin superfamily. *J Clin Invest.* 2004; 114:1741–1751. [PubMed: 15599399]
10. Toth C, Rong LL, Yang C, Martinez J, Song F, Ramji N, Brussee V, Liu W, Durand J, Nguyen MD, Schmidt AM, Zochodne DW. Receptor for advanced glycation end products (RAGEs) and experimental diabetic neuropathy. *Diabetes.* 2008; 57:1002–1017. [PubMed: 18039814]
11. Cameron NE, Cotter MA, Jack AM, Basso MD, Hohman TC. Protein kinase C effects on nerve function, perfusion, Na(+), K(+)-ATPase activity and glutathione content in diabetic rats. *Diabetologia.* 1999; 42:1120–1130. [PubMed: 10447525]
12. Purves T, Middlemas A, Agthong S, Jude EB, Boulton AJ, Fernyhough P, Tomlinson DR. A role for mitogen-activated protein kinases in the etiology of diabetic neuropathy. *FASEB J.* 2001; 15:2508–2514. [PubMed: 11689477]
13. Du Y, Tang J, Li G, Berti-Mattera L, Lee CA, Bartkowski D, Gale D, Monahan J, Niesman MR, Alton G, Kern TS. Effects of p38 MAPK inhibition on early stages of diabetic retinopathy and sensory nerve function. *Invest Ophthalmol Vis Sci.* 2010; 51:2158–2164. [PubMed: 20071676]
14. Kellogg AP, Wiggin TD, Larkin DD, Hayes JM, Stevens MJ, Pop-Busui R. Protective effects of cyclooxygenase-2 gene inactivation against peripheral nerve dysfunction and intraepidermal nerve fiber loss in experimental diabetes. *Diabetes.* 2007; 56:2997–3005. [PubMed: 17720896]
15. Stavniichuk R, Drel VR, Shevalye H, Varenjuk I, Stevens MJ, Nadler JL, Obrosova IG. Role of 12/15-lipoxygenase in nitrosative stress and peripheral prediabetic and diabetic neuropathies. *Free Radic Biol Med.* 2010; 49:1036–1045. [PubMed: 20599608]
16. Nakae M, Kamiya H, Naruse K, Horio N, Ito Y, Mizubayashi R, Hamada Y, Nakashima E, Akiyama N, Kobayashi Y, Watarai A, Kimura N, Horiguchi M, Tabata Y, Oiso Y, Nakamura J. Effects of basic fibroblast growth factor on experimental diabetic neuropathy in rats. *Diabetes.* 2006; 55:1470–1477. [PubMed: 16644707]
17. Shibata T, Naruse K, Kamiya H, Kozakae M, Kondo M, Yasuda Y, Nakamura N, Ota K, Tosaki T, Matsuki T, Nakashima E, Hamada Y, Oiso Y, Nakamura J. Transplantation of bone marrow-derived mesenchymal stem cells improves diabetic polyneuropathy in rats. *Diabetes.* 2008; 57:3099–3107. [PubMed: 18728233]
18. McGuire JF, Rouen S, Siegfried E, Wright DE, Dobrowsky RT. Caveolin-1 and altered neuregulin signaling contribute to the pathophysiological progression of diabetic peripheral neuropathy. *Diabetes.* 2009; 58:2677–2686. [PubMed: 19675140]
19. Nagamatsu M, Nickander KK, Schmelzer JD, Raya A, Wittrock DA, Tritschler H, Low PA. Lipoic acid improves nerve blood flow, reduces oxidative stress, and improves distal nerve conduction in experimental diabetic neuropathy. *Diabetes Care.* 1995; 18:1160–1167. [PubMed: 7587852]
20. Cameron NE, Tuck Z, McCabe L, Cotter MA. Effect of the hydroxyl radical scavenger, dimethylthiourea, on peripheral nerve tissue perfusion, conduction velocity and nociception in experimental diabetes. *Diabetologia.* 2001; 44:1161–1169. [PubMed: 11596672]
21. Coppey LJ, Gellett JS, Davidson EP, Dunlap JA, Lund DD, Yorek MA. Effect of antioxidant treatment of streptozotocin-induced diabetic rats on endoneurial blood flow, motor nerve conduction velocity, and vascular reactivity of epineurial arterioles of the sciatic nerve. *Diabetes.* 2001; 50:1927–1937. [PubMed: 11473057]
22. Obrosova IG, Li F, Abatan OI, Forsell MA, Komjati K, Pacher P, Szabo C, Stevens MJ. Role of poly(ADP-ribose) polymerase activation in diabetic neuropathy. *Diabetes.* 2004; 53:711–720. [PubMed: 14988256]
23. Obrosova IG, Drel VR, Oltman CL, Mashtalir N, Tibrewala J, Groves JT, Yorek MA. Role of nitrosative stress in early neuropathy and vascular dysfunction in streptozotocin-diabetic rats. *Am J Physiol Endocrinol Metab.* 2007; 293:E1645–E1655. [PubMed: 17911342]
24. Obrosova IG, Xu W, Lyzogubov VV, Ilnytska O, Mashtalir N, Varenjuk I, Pavlov IA, Zhang J, Slusher B, Drel VR. PARP inhibition or gene deficiency counteracts intraepidermal nerve fiber loss and neuropathic pain in advanced diabetic neuropathy. *Free Radic Biol Med.* 2008; 44:972–981. [PubMed: 17976390]

25. Drel VR, Lupachyk S, Shevalye H, Vareniuk I, Xu W, Zhang J, Delamere NA, Shahidullah M, Slusher B, Obrosova IG. New therapeutic and biomarker discovery for peripheral diabetic neuropathy: PARP inhibitor, nitrotyrosine, and tumor necrosis factor- α . *Endocrinology*. 2010; 151:2547–2555. [PubMed: 20357221]
26. Obrosova IG. Diabetes and the peripheral nerve. *Biochim Biophys Acta*. 2009; 1792:931–940. [PubMed: 19061951]
27. Cheng C, Zochodne DW. Sensory neurons with activated caspase-3 survive long-term experimental diabetes. *Diabetes*. 2003; 52:2363–2371. [PubMed: 12941777]
28. Coppey LJ, Gellett JS, Davidson EP, Yorek MA. Preventing superoxide formation in epineurial arterioles of the sciatic nerve from diabetic rats restores endothelium-dependent vasodilation. *Free Radic Res*. 2003; 37:33–40. [PubMed: 12653215]
29. Schmeichel AM, Schmelzer JD, Low PA. Oxidative injury and apoptosis of dorsal root ganglion neurons in chronic experimental diabetic neuropathy. *Diabetes*. 2003; 52:165–171. [PubMed: 12502508]
30. Szabo C, Zanchi A, Komjati K, Pacher P, Krolewski AS, Quist WC, LoGerfo FW, Horton ES, Veves A. Poly(ADP-ribose) polymerase is activated in subjects at risk of developing type 2 diabetes and is associated with impaired vascular reactivity. *Circulation*. 2002; 106:2680–2686. [PubMed: 12438293]
31. Jagtap P, Szabo C. Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. *Nat Rev Drug Discov*. 2005; 4:421–440. [PubMed: 15864271]
32. Kauppinen TM, Chan WY, Suh SW, Wiggins AK, Huang EJ, Swanson RA. Direct phosphorylation and regulation of poly(ADP-ribose) polymerase-1 by extracellular signal-regulated kinases 1/2. *Proc Natl Acad Sci USA*. 2006; 103:7136–7141. [PubMed: 16627622]
33. Wang Y, Schmeichel AM, Iida H, Schmelzer JD, Low PA. Ischemia-reperfusion injury causes oxidative stress and apoptosis of Schwann cell in acute and chronic experimental diabetic neuropathy. *Antioxid Redox Signal*. 2005; 7:1513–1520. [PubMed: 16356115]
34. Obrosova IG, Ilnytska O, Lyzogubov VV, Pavlov IA, Mashtalir N, Nadler JL, Drel VR. High-fat diet induced neuropathy of pre-diabetes and obesity: effects of "healthy" diet and aldose reductase inhibition. *Diabetes*. 2007; 56:2598–2608. [PubMed: 17626889]
35. Drel VR, Xu W, Zhang J, Kador PF, Ali TK, Shin J, Julius U, Slusher B, El-Remessy AB, Obrosova IG. Poly(ADP-ribose) polymerase inhibition counteracts cataract formation and early retinal changes in streptozotocin-diabetic rats. *Invest Ophthalmol Vis Sci*. 2009; 50:1778–1790. [PubMed: 19098320]
36. Szabo C, Biser A, Benko R, Bottinger E, Susztak K. Poly(ADP-ribose) polymerase inhibitors ameliorate nephropathy of type 2 diabetic Leprdb/db mice. *Diabetes*. 2006; 55:3004–3012. [PubMed: 17065336]
37. Low PA, Nickander KK, Tritschler HJ. The roles of oxidative stress and antioxidant treatment in experimental diabetic neuropathy. *Diabetes*. 1997; 46 Suppl 2:S38–S42. [PubMed: 9285497]
38. van Dam PS, van Asbeck BS, Van Oirschot JF, Biessels GJ, Hamers FP, Marx JJ. Glutathione and alpha-lipoate in diabetic rats: nerve function, blood flow and oxidative state. *Eur J Clin Invest*. 2001; 31:417–424. [PubMed: 11380593]
39. Prabhakar S, Starnes J, Shi S, Lonis B, Tran R. Diabetic nephropathy is associated with oxidative stress and decreased renal nitric oxide production. *J Am Soc Nephrol*. 2007; 18:2945–2952. [PubMed: 17928507]
40. Akude E, Zherebitskaya E, Roy Chowdhury SK, Girling K, Fernyhough P. 4-Hydroxy-2-nonenal induces mitochondrial dysfunction and aberrant axonal outgrowth in adult sensory neurons that mimics features of diabetic neuropathy. *Neurotox Res*. 2010; 17:28–38. [PubMed: 19557324]
41. Kokubo J, Nagatani N, Hiroki K, Kuroiwa K, Watanabe N, Arai T. Mechanism of destruction of microtubule structures by 4-hydroxy-2-nonenal. *Cell Struct Funct*. 2008; 33:51–59. [PubMed: 18360009]
42. Petersen DR, Doorn JA. Reactions of 4-hydroxynonenal with proteins and cellular targets. *Free Radic Biol Med*. 2004; 37:937–945. [PubMed: 15336309]

43. Mezzetti A, Cipollone F, Cuccurullo F. Oxidative stress and cardiovascular complications in diabetes: isoprostanes as new markers on an old paradigm. *Cardiovasc Res.* 2000; 47:475–488. [PubMed: 10963721]
44. Shimoike T, Inoguchi T, Umeda F, Nawata H, Kawano K, Ochi H. The meaning of serum levels of advanced glycosylation end products in diabetic nephropathy. *Metabolism.* 2000; 49:1030–1035. [PubMed: 10954022]
45. Ziegler D, Sohr CG, Nourooz-Zadeh J. Oxidative stress and antioxidant defense in relation to the severity of diabetic polyneuropathy and cardiovascular autonomic neuropathy. *Diabetes Care.* 2004; 27:2178–2183. [PubMed: 15333481]
46. Hoeldtke RD, Bryner KD, Hoeldtke ME, Christie I, Ganser G, Hobbs G, Riggs J. Sympathetic sudomotor disturbance in early type 1 diabetes mellitus is linked to lipid peroxidation. *Metabolism.* 2006; 55:1524–1531. [PubMed: 17046556]
47. Ceriello A, Esposito K, Ihnat M, Thorpe J, Giugliano D. Long-term glycemic control influences the long-lasting effect of hyperglycemia on endothelial function in type 1 diabetes. *J Clin Endocrinol Metab.* 2009; 94:2751–2756. [PubMed: 19491221]
48. Obrosova IG, Van HC, Fathallah L, Cao X, Stevens MJ, Greene DA. Evaluation of alpha(1)-adrenoceptor antagonist on diabetes-induced changes in peripheral nerve function, metabolism, and antioxidative defense. *FASEB J.* 2000; 14:1548–1558. [PubMed: 10928989]
49. Hounsom L, Corder R, Patel J, Tomlinson DR. Oxidative stress participates in the breakdown of neuronal phenotype in experimental diabetic neuropathy. *Diabetologia.* 2001; 44:424–428. [PubMed: 11357471]
50. Selvarajah D, Wilkinson ID, Emery CJ, Harris ND, Shaw PJ, Witte DR, Griffiths PD, Tesfaye S. Early involvement of the spinal cord in diabetic peripheral neuropathy. *Diabetes Care.* 2006; 29:2664–2669. [PubMed: 17130202]
51. Wang G, Pierangeli SS, Papalardo E, Ansari GA, Khan MF. Markers of oxidative and nitrosative stress in systemic lupus erythematosus: correlation with disease activity. *Arthritis Rheum.* 2010; 62:2064–2072. [PubMed: 20201076]
52. Varenuk I, Pavlov IA, Obrosova IG. Inducible nitric oxide synthase gene deficiency counteracts multiple manifestations of peripheral neuropathy in a streptozotocin-induced mouse model of diabetes. *Diabetologia.* 2008; 51:2126–2133. [PubMed: 18802679]
53. Chowdhury SK, Zhrebetskaya E, Smith DR, Akude E, Chattopadhyay S, Jolivald CG, Calcutt NA, Fernyhough P. Mitochondrial respiratory chain dysfunction in dorsal root ganglia of streptozotocin-induced diabetic rats and its correction by insulin treatment. *Diabetes.* 2010; 59:1082–1091. [PubMed: 20103706]
54. Goss JR, Goins WF, Lacomis D, Mata M, Glorioso JC, Fink DJ. Herpes simplex-mediated gene transfer of nerve growth factor protects against peripheral neuropathy in streptozotocin-induced diabetes in the mouse. *Diabetes.* 2002; 51:2227–2232. [PubMed: 12086954]
55. Chattopadhyay M, Mata M, Goss J, Wolfe D, Huang S, Glorioso JC, Fink DJ. Prolonged preservation of nerve function in diabetic neuropathy in mice by herpes simplex virus-mediated gene transfer. *Diabetologia.* 2007; 50:1550–1558. [PubMed: 17508196]
56. Drel VR, Mashtalir N, Ilnytska O, Shin J, Li F, Lyzogubov VV, Obrosova IG. The leptin-deficient (ob/ob) mouse: a new animal model of peripheral neuropathy of type 2 diabetes and obesity. *Diabetes.* 2006; 55:3335–3343. [PubMed: 17130477]
57. Vincent AM, Hayes JM, McLean LL, Vivekanandan-Giri A, Pennathur S, Feldman EL. Dyslipidemia-induced neuropathy in mice: the role of oxLDL/LOX-1. *Diabetes.* 2009; 58:2376–2385. [PubMed: 19592619]
58. Varenuk I, Pacher P, Pavlov IA, Drel VR, Obrosova IG. Peripheral neuropathy in mice with neuronal nitric oxide synthase gene deficiency. *Int J Mol Med.* 2009; 23:571–580. [PubMed: 19360314]
59. Pacher P, Szabo C. Role of the peroxynitrite-poly(ADP-ribose) polymerase pathway in human disease. *Am J Pathol.* 2008; 173:2–13. [PubMed: 18535182]
60. Ha HC, Hester LD, Snyder SH. Poly(ADP-ribose) polymerase-1 dependence of stress-induced transcription factors and associated gene expression in glia. *Proc Natl Acad Sci USA.* 2002; 99:3270–3275. [PubMed: 11854472]

61. Drel VR, Xu W, Zhang J, Pavlov IA, Shevalye H, Slusher B, Obrosova IG. Poly(Adenosine 5'-diphosphate-ribose) polymerase inhibition counteracts multiple manifestations of experimental type 1 diabetic nephropathy. *Endocrinology*. 2009; 150:5273–5283. [PubMed: 19854869]
62. Veres B, Radnai B, Gallyas F Jr, Varbiro G, Berente Z, Osz E, Sumegi B. Regulation of kinase cascades and transcription factors by a poly(ADP-ribose) polymerase-1 inhibitor, 4-hydroxyquinazoline, in lipopolysaccharide-induced inflammation in mice. *J Pharmacol Exp Ther*. 2004; 310:247–255. [PubMed: 14999056]
63. Garcia SF, Virag L, Jagtap P, Szabo E, Mabley JG, Liaudet L, Marton A, Hoyt DG, Murthy KG, Salzman AL, Southan GJ, Szabo C. Diabetic endothelial dysfunction: the role of poly(ADP-ribose) polymerase activation. *Nat Med*. 2001; 7:108–113. [PubMed: 11135624]
64. Pacher P, Liaudet L, Soriano FG, Mabley JG, Szabo E, Szabo C. The role of poly(ADP-ribose) polymerase activation in the development of myocardial and endothelial dysfunction in diabetes. *Diabetes*. 2002; 51:514–521. [PubMed: 11812763]
65. Zheng L, Szabo C, Kern TS. Poly(ADP-ribose) polymerase is involved in the development of diabetic retinopathy via regulation of nuclear factor-kappaB. *Diabetes*. 2004; 53:2960–2967. [PubMed: 15504977]
66. Obrosova IG, Drel VR, Pacher P, Ilnytska O, Wang ZQ, Stevens MJ, Yorek MA. Oxidative-nitrosative stress and poly(ADP-ribose) polymerase (PARP) activation in experimental diabetic neuropathy: the relation is revisited. *Diabetes*. 2005; 54:3435–3441. [PubMed: 16306359]
67. Li F, Szabo C, Pacher P, Southan GJ, Abatan OI, Charniauskaya T, Stevens MJ, Obrosova IG. Evaluation of orally active poly(ADP-ribose) polymerase inhibitor in streptozotocin-diabetic rat model of early peripheral neuropathy. *Diabetologia*. 2004; 47:710–717. [PubMed: 15298348]
68. Wolin MS, Ahmad M, Gupte SA. Oxidant and redox signaling in vascular oxygen sensing mechanisms: basic concepts, current controversies, and potential importance of cytosolic NADPH. *Am J Physiol Lung Cell Mol Physiol*. 2005; 289:L159–L173. [PubMed: 16002998]
69. Wolin MS, Ahmad M, Gao Q, Gupte SA. Cytosolic NAD(P)H regulation of redox signaling and vascular oxygen sensing. *Antioxid Redox Signal*. 2007; 9:671–678. [PubMed: 17511583]
70. Price SA, Gardiner NJ, Duran-Jimenez B, Zeef LA, Obrosova IG, Tomlinson DR. Thioredoxin interacting protein is increased in sensory neurons in experimental diabetes. *Brain Res*. 2006; 1116:206–214. [PubMed: 16938273]
71. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, Ashworth A, Carmichael J, Kaye SB, Schellens JH, de Bono JS. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med*. 2009; 361:123–134. [PubMed: 19553641]
72. Weston VJ, Oldreive CE, Skowronska A, Oscier DG, Pratt G, Dyer MJ, Smith G, Powell JE, Rudzki Z, Kearns P, Moss PA, Taylor AM, Stankovic T. The PARP inhibitor olaparib induces significant killing of ATM-deficient lymphoid tumor cells in vitro and in vivo. *Blood*. 2010; 116:4578–4587. [PubMed: 20739657]

Fig. 1. Representative microphotographs of fluorescent immunostaining of rat sciatic nerve for poly(ADP-ribose) (*left*); DAPI (*center*), poly(ADP-ribose) and DAPI (*right*). Magnification $\times 125$.

Fig. 2.

Representative Western blot analyses of sciatic nerve (A) and spinal cord (C) poly(ADP-ribose)ylated proteins, and poly(ADP-ribose)ylated protein content (densitometry, B and D) in control and diabetic rats maintained with or without PARP inhibitor treatment. C – control; D – diabetic. Mean $\pm$ SEM, n = 6 per group. * p < 0.05 vs control group; #, ## p < 0.05 and < 0.01 vs untreated diabetic group.

Fig. 3.

Representative microphotographs of poly(ADP-ribose) fluorescence (A) and relative fluorescence counts per cell area (B) in dorsal root ganglion neurons (white arrows) and non-neuronal cells (green arrows) in control and diabetic rats maintained with or without PARP inhibitor treatment. C – control; D – diabetic. Mean $\pm$ SEM, n = 53–177 per group for neurons, and n = 45–105 per group for non-neuronal cells. ** p < 0.01 vs control group; ## p < 0.01 vs untreated diabetic group.

Fig. 4. Urinary isoprostane (A) and 8-hydroxy-2'-deoxyguanosine (B) excretion in control and diabetic rats maintained with or without PARP inhibitor treatment. C – control; D – diabetic. Mean $\pm$ SEM, $n = 5-8$ per group. *, ** $p < 0.05$ and < 0.01 vs control group; # $p < 0.05$ vs untreated diabetic group.

Fig. 5. Representative Western blot analyses of sciatic nerve (A) and spinal cord (C) 4-hydroxynonenal protein adducts, and 4-hydroxynonenal protein adduct content (densitometry, B and D), in control and diabetic rats maintained with or without PARP inhibitor treatment. C – control; D – diabetic. Mean $\pm$ SEM, $n = 6-8$ per group. ** $p < 0.01$ vs control group; #,## $p < 0.05$ and < 0.01 vs untreated diabetic group.

Fig. 6. Representative microphotographs of 4-hydroxynonenal protein adduct fluorescence (A) and relative fluorescence counts per cell area (B) in dorsal root ganglion neurons in control and diabetic rats maintained with or without PARP inhibitor treatment. C – control; D – diabetic. Mean ± SEM, n = 43–110 per group, ** p < 0.01 vs control group; ## p < 0.01 vs untreated diabetic group.

Fig. 7.

(A,B) Representative microphotographs of fluorescent immunostaining [or fluorescent staining with isolectin Alexa Fluor(R) 594 conjugate, A (center)] of diabetic rat sciatic nerve for (A) 4-hydroxynonenal protein adducts (left); vascular endothelium (center), and 4-hydroxynonenal protein adducts and vascular endothelium (right); and (B) 4-hydroxynonenal protein adducts (left); S-100 (center), 4-hydroxynonenal protein adducts and S-100 (right); Magnification $\times 125$.

(C) Representative microphotographs of fluorescent immunostaining of ventral horn motor neurons of diabetic rat spinal cord for 4-hydroxynonenal protein adducts (left); the DNA-

binding, neuron-specific protein NeuN (*center*), and 4-hydroxynonenal protein adducts and NeuN (*right*), Magnification $\times 100$.

(D,E) Representative microphotographs of fluorescent immunostaining of white matter of diabetic rat spinal cord for (D) 4-hydroxynonenal protein adducts (*left*); the oligodendrocyte marker adenomatous polyposis coli -Ab7 (*center*), 4-hydroxynonenal protein adducts and adenomatous polyposis coli -Ab7 (*right*), and (E) 4-hydroxynonenal protein adducts (*left*); the astrocyte marker glial fibrillary acidic protein (*center*), 4-hydroxynonenal protein adducts and glial fibrillary acidic protein (*right*). Magnification $\times 125$.

(F) Representative microphotographs of fluorescent immunostaining of diabetic rat dorsal root ganglia for 4-hydroxynonenal protein adducts (*left*); glutamine synthetase (*center*), 4-hydroxynonenal protein adducts and glutamine synthetase (*right*). Magnification $\times 80$. 4-HNE – 4-hydroxynonenal adducts; GFAP – glial fibrillary acidic protein; GS – glutamine synthetase; APC - adenomatous polyposis coli.

Table 1

Initial and final body weights and blood glucose concentrations in control and diabetic rats maintained with or without 1,5-isoquinolinediol treatment

Variable Group	Body weight (g)		Blood glucose (mmol/l)	
	Initial	Final	Initial	Final
Control	271 ± 2.8	513 ± 27	5.5 ± 0.07	5.7 ± 0.4
Control + ISO	275 ± 3.4	508 ± 15	6.0 ± 0.16	5.5 ± 0.2
Diabetic	268 ± 3.6	323 ± 11 **	22.4 ± 1.27 **	21.5 ± 1.3 **
Diabetic + ISO	267 ± 3.7	309 ± 18 **	23.2 ± 0.98 **	22.7 ± 1.1 **

Data are expressed as Means ± SEM, n = 10 per group.

**
p < 0.01 vs controls. ISO - 1,5-isoquinolinediol.

Table 2

Motor and sensory nerve conduction velocities, thermal response latencies, and mechanical and tactile withdrawal thresholds, in control and diabetic rats maintained with or without 1,5-isoquinolinediol treatment

Groups Variable	Control	Control+ISO	Diabetic	Diabetic+ISO
Baseline (prior to induction of STZ-diabetes)				
MNCV, ms ⁻¹	53.4 ± 0.8			
SNCV, ms ⁻¹	39.5 ± 0.5			
Thermal response latency, s	11.0 ± 0.4			
Mechanical withdrawal threshold, g	134 ± 10			
Tactile withdrawal threshold, g	15.0 ± 1.6			
2-wk time point (prior to ISO administration)				
MNCV, ms ⁻¹	54.1 ± 0.7		45.2 ± 0.5**	
SNCV, ms ⁻¹	39.7 ± 0.4		31.5 ± 0.3**	
Thermal response latency, s	10.8 ± 0.5		6.5 ± 0.4**	
Mechanical withdrawal threshold, g	128 ± 9		101 ± 7**	
Tactile withdrawal threshold, g	15.6 ± 1.2		15.2 ± 0.9	
12-wk time point (final measurements)				
MNCV, ms ⁻¹	55.5 ± 1.1	54.3 ± 1.2	46.1 ± 0.4**	54.7 ± 1.3##
SNCV, ms ⁻¹	40.6 ± 0.3	39.6 ± 0.4	32.7 ± 0.5**	39.4 ± 0.5##
Thermal response latency, s	10.5 ± 0.5	10.9 ± 0.3	16.0 ± 0.9**	12.4 ± 0.6*##
Mechanical withdrawal threshold, g	130 ± 13	133 ± 9	90 ± 7**	127 ± 7**
Tactile withdrawal threshold, g	14.8 ± 1.7	13.9 ± 2.0	6.2 ± 0.6**	10.2 ± 1.2*##

Data are expressed as Mean ± SEM, n = 7–10 per group.

*, ** p < 0.05 and < 0.01 vs controls;

#, ## p < 0.05 and < 0.01 vs untreated diabetic group. ISO - 1,5 - isoquinolinediol.