Induction of hemeoxygenase-1 reduces glomerular injury and apoptosis in diabetic spontaneously hypertensive rats

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Elmarakby AA, Faulkner J, Bahan B, Saleh MA, Sullivan JC. Induction of hemeoxygenase-1 reduces glomerular injury and apoptosis in diabetic spontaneously hypertensive rats. Am J Physiol Renal Physiol 302: F791–F800, 2012. First published December 28, 2011; doi:10.1152/ajprenal.00472.2011.—Induction of hemeoxygenase-1 (HO-1) lowers blood pressure and reduces organ damage in hypertensive animal models; however, a potential protective role for HO-1 induction against diabetic-induced glomerular injury remains unclear. We hypothesize that HO-1 induction will protect against diabetes-induced glomerular injury by maintaining glomerular integrity and inhibiting renal apoptosis, inflammation, and oxidative stress. Diabetes was induced with streptozotocin in spontaneously hypertensive rats (SHR) as a model where the coexistence of hypertension and diabetes aggravates the progression of diabetic renal injury. Control and diabetic SHR were randomized to receive vehicle or the HO-1 inducer cobalt protoporphyrin (CoPP). Glomerular albumin permeability was significantly greater in diabetic SHR compared with control, consistent with an increase in apoptosis and decreased glomerular nephrin and α2β1-integrin protein expression in diabetic SHR. CoPP significantly reduced albumin permeability and apoptosis and restored nephrin and α2β1-integrin protein expression levels in diabetic SHR. Glomerular injury in diabetic SHR was also associated with increases in NF-κB-induced inflammation and oxidative stress relative to vehicle-treated SHR, and CoPP significantly blunted diabetes-induced increases in glomerular inflammation and oxidative stress in diabetic SHR. These effects were specific to exogenous stimulation of HO-1, since incubation with the HO inhibitor stannous mesoporphyrin alone did not alter glomerular inflammatory markers or oxidative stress yet was able to prevent CoPP-mediated decreases in these parameters. These data suggest that induction of HO-1 reduces diabetic-induced glomerular injury and apoptosis and these effects are associated with decreased NF-κB-induced inflammation and oxidative stress.

Hypertension; CoPP; glomeruli; albumin permeability; NF-κB; ICAM-1

Diabetes mellitus is a growing public health burden worldwide (10, 26), and the number of patients with diabetes is expected to increase 50–70% within the next 25 years (36, 42). Diabetic nephropathy (DN) is a progressive kidney disease that affects ~35% of type 1 and type 2 diabetic patients and is one of the primary causes of renal failure (9, 14). As DN progresses, there is an elevation in blood pressure and decline in renal function, resulting in end-stage renal disease (35). Although hyperglycemia and hypertension have both been implicated in the pathogenesis of DN, the etiology of DN is complex and multifactorial. Hypertension is commonly found to coexist in patients with diabetes, and elevations in blood pressure negatively impact renal function (25). This study employs a unique model to investigate the effects of hyperglycemia on a hypertensive background by inducing diabetes in spontaneously hypertensive rats (SHR), a genetic model of essential hypertension.

An early hallmark of DN is the appearance of glomerular injury and proteinuria (37). Although the mechanisms of increased proteinuria in diabetes have not been clearly investigated, a defect in the glomerular filtration barrier could be a potential mechanism. The glomerular filtration barrier is formed by fenestrated vascular endothelium, the glomerular basement membrane, and visceral epithelial podocytes which are separated by slit diaphragms. Podocytes attach to the glomerular basement membrane through adhesion proteins, mainly α2β1-integrin, and to the filtration slit molecules including nephrin to maintain proper barrier function and prevent the loss of protein (21, 35). In diabetes, the defect in the glomerular filtration barrier is mainly attributed to damage to the filtration slit formed by glomerular podocytes and/or down-regulation of α2β1-integrin, leading to podocyte loss (21, 37). Nephrin is a transmembrane protein that plays a crucial role in maintaining the integrity of the glomerular filtration barrier, and previous studies have shown decreased glomerular nephrin expression with increased levels of urinary nephrin excretion in diabetes, which may contribute to podocyte loss (4, 21, 37, 38). It is well known that the number of podocyte decreases in diabetic patients and diabetic animal models (22, 43, 45), and renal apoptosis has been implicated in podocyte loss under diabetic conditions (22, 45, 49).

In recent years, there has been an increased focus on a potential causative role for inflammation in the development and progression of DN. The pathogenesis of both DN and hypertension is associated with glomerular damage, and the mechanisms have been linked to inflammation (5, 34). Increased macrophage infiltration and overproduction of leukocyte adhesion molecules have been demonstrated in the kidneys of diabetic humans and experimental animal models (8, 31). Activated inflammatory cells further increase cytokine release, leading to fibrosis, matrix deposition, and progressive renal injury (8, 46). In particular, it is now well established that macrophage infiltration and activation of a NF-κB inflammatory signaling pathway play a crucial role in the progression of renal injury via the activation of proinflammatory molecules such as monocyte chemottractant protein (MCP)-1 and ICAM-1 (14). Although numerous studies suggest a role for hyperglycemia in the increase in vascular inflammation in diabetic animal models (7, 13, 17, 33, 39), the majority of these diabetic animals remain normotensive. The coexistence of hypertension and diabetes in the current animal model exaggerates the incidence of glomerular injury and better reflects the clinical setting.
Heme oxygenase (HO) is the primary pathway for heme catabolism, driving the conversion of heme to biliverdin, iron, and carbon monoxide. There are two isoenzymes of HO: HO-1 and HO-2. HO-1 is inducible and is upregulated in response to hypoxia, oxidative stress, ischemia, and cytokines (1, 47). HO-2 is constitutively active and accounts for most HO activity in the normal state (47). In streptozotocin-induced diabetic Sprague-Dawley rats, HO-1 has been found to be induced in podocytes, protecting against apoptosis (22). Induction of HO-1 also reduces blood pressure and inflammation in diabetic spontaneously hypertensive rats (SHR) (6), which led us to hypothesize that HO-1 induction will reduce glomerular injury and apoptosis in diabetic SHR via anti-inflammatory mechanisms.

MATERIALS AND METHODS

Animals. All procedures using animals were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and Georgia Health Sciences University guidelines. Ten-week-old male SHR (Charles River) were used in this study. Diabetes was induced by a single injection of streptozotocin (60 mg/kg iv, Sigma, St. Louis, MO), and rats were supplemented with sustained-release insulin implants (sc, Lanshin) to maintain blood glucose levels within 450–550 mg/dl. Blood glucose was tested weekly using a glucometer, and all experiments were performed after the induction of diabetes. Control and diabetic SHR were treated with vehicle (0.1 M NaOH, pH 8.3) or the HO-1 inducer cobalt protoporphyrin (CoPP; 50 μg/100 g body wt sc) weekly for 6 wk immediately after the induction of diabetes (n = 8–10/group). Systolic blood pressure was measured weekly using the tail-cuff method (IITC Life Science, Woodland Hills, CA) (12, 12a). After 6 wk, rats were placed in metabolic cages (Nalgene Rochester, NY) for 24-h urine collection. Urinary MCP-1 (BD Biosciences, Bedford, MA) was measured as an index of inflammation. Urinary protein (Bio-Rad, Hercules, CA), albumin, and nephrin (Exocell, Philadelphia, PA) excretions were determined as indices of renal injury. Urinary collagen IV excretion was measured using a commercial available kit (Exocell). Urinary 8-hydroxy deoxyguanosine (8-OHdG; Northwest, WA) excretion levels were assessed as a marker of oxidative stress.

Isolation of glomeruli and albumin permeability. Glomeruli were isolated from the kidney by a gradual sieving technique as previously described (37). Half of the isolated glomeruli were used to determine glomerular albumin permeability as previously described (37). Briefly, images of 10–15 glomeruli suspended in 5% BSA were captured using an inverted microscope before and after a medium change to 1% BSA.

Nephrin and caspase 3 immunofluorescence. Additional glomeruli were frozen for immunofluorescent detection of nephrin and caspase 3 levels. Glomeruli were fixed using paraformaldehyde, washed with PBS, and incubated for 1 h with normal goat serum in PBS as previously described (37). Slides were then incubated overnight at 4°C with goat anti-nephrin (Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-caspase 3 (Cell Signaling Technology, Danvers, MA), or mouse anti-synaptopodin (ARP American Research Products, Belmont, MA) primary antibodies. On the second day, washing and blocking procedures were repeated before incubation of slides for 1 h with Texas red- and Oregon green-labeled secondary antibodies (Molecular Probes, Carlsbad, CA). Slides were then examined by confocal microscopy (LSM 510, Carl Zeiss, Oberkochen, Germany). Images were collected from five grids from each slide (n = 3/group).

Terminal dUTP nick end-labeling (TUNEL) analysis. Terminal dUTP nick end-labeling (TUNEL) analysis was performed using the ApopTag Fluorescein In-Situ Apoptosis Detection Kit (Millipore, Temecula, CA) according to the manufacturer’s specifications. Briefly, kidney sections from each rat group were fixed using paraformaldehyde and an ethanol/acetic acid solution (2:1). Sections were incubated with terminal deoxynucleotidyl transferase followed by incubation with an anti-digoxigenin conjugate. Propidium iodide (1 μg/ml) was added as a nuclear counterstain. Coverslips were applied using Vectashield mounting medium for fluorescence (Vector Laboratories, Burlingame, CA). Images were obtained using confocal microscopy (LSM 510, Carl Zeiss) with ×100 magnification. Whole kidney sections were scanned for positive green fluorescent cells as an indicator of apoptosis.

Homogenization of the renal cortex for protein expression using Western blot analysis. Isolated glomeruli and the kidney cortex were homogenized in RIPA buffer supplemented with inhibitors for proteases and phosphatases as previously described (11). Protein concentrations were determined by a standard Bradford assay (Bio-Rad). Glomerular and cortical samples were separated by SDS-PAGE as previously described (12). Gels were then transferred onto nitrocellulose membranes and incubated with specific antibodies. The primary antibodies used were rabbit IKKα, phospho-IKKα (ser180/IKKβ (ser181), caspase 3 active fragments (Cell Signaling Technology, nphrin, α2,6-β1,4 integrin, β1-integrin (Santa Cruz Biotechnology), HO-1, HO-2 (EMD Biosciences, San Diego, CA), and mouse β-actin (Sigma). These antibodies were detected with a horseradish peroxidase-conjugated secondary antibody and ECL chemiluminescence (Amersham Biosciences, Buckinghamshire, UK). The intensity of immunoreactivity was measured by densitometry, and β-actin was used to verify equal protein loading.

Renal NF-κB and soluble ICAM-1 assays. Renal cortical samples were used to prepare whole-cell extracts using a nuclear extract kit (Active Motif, Carlsbad, CA). Cortical whole-cell extracts were used for the determination of NF-κB activity using a TransAM P65-NF-κB transcription factor assay kit (Active Motif). Glomerular soluble ICAM-1 (sICAM-1) levels were determined using a commercially available kit (R&D Systems, Minneapolis, MN).

Renal histopathology. In a separate set of rats (n = 3/group), kidneys were perfused with a 10% formalin solution and then paraffin embedded and cut into 4- to 5-μm sections. Kidney sections were used for immunohistochemical evaluation of fibronectin and collagen as markers of renal fibrosis and F4/80 as a marker for activated macrophages (anti-F4/80 antibody from Santa Cruz Biotechnology). Five microscopic images of the kidney cortex per rat were randomly taken at ×400 magnification.

Ex vivo treatment of isolated glomeruli with a HO inhibitor and/or inducer. Glomeruli from control and diabetic SHR and incubated for 2 h at 37°C with the HO inhibitor stannous mesoporphyrin (SnMP; 20 mM), CoPP (10 mM), or both SnMP and CoPP (n = 4–6/group). Glomerular superoxide levels was determined using lucigenin chemiluminescence and dihydroethidine (DHE) staining as previously described (5). Glomerular ICAM-1 expression was assessed by Western blot analysis (antibody from R&D Systems).

Data analysis. Statistical analyses were performed using Prism software (GraphPad, San Diego, CA). Data are reported as means ± SE and were analyzed using one-way ANOVA followed by Tukey’s post hoc test. For all comparisons, P < 0.05 was considered significant.

RESULTS

Blood pressure, blood glucose, and proteinuria. As shown in Fig. 1, A and B, induction of diabetes did not significantly affect renal HO-1 or HO-2 expression in SHR; however, CoPP treatment significantly increased renal HO-1 without affecting HO-2 expression in both control and diabetic SHR. Following the induction of diabetes with streptozotocin, blood glucose levels were significantly elevated in diabetic vs. control SHR (513 ± 30 vs. 161 ± 11 mg/dl, respectively; P < 0.0002). Induction of HO-1 with CoPP reduced blood glucose in control
and diabetic SHR (138 ± 7 and 442 ± 8 mg/dl, respectively); however, this decrease only reached significance in diabetic SHR (P < 0.05). Blood glucose levels in diabetic SHR treated with CoPP remained significantly higher than control SHR (442 ± 8 vs. 161 ± 11 mg/dl; P < 0.005). Systolic blood pressure was comparable in control and diabetic SHR (diabetic SHR: 200 ± 4; control SHR: 192 ± 5 mm/Hg; P < 0.2); however, induction of HO-1 with CoPP reduced systolic blood pressure in both control and diabetic SHR (184 ± 3 & 184 ± 12 mmHg, respectively). Diabetic SHR also exhibited significantly greater proteinuria compared with control SHR (P < 0.05). CoPP treatment lowered proteinuria in control SHR and abolished the elevation in proteinuria in diabetic SHR (P < 0.05) (Fig. 2A). Albuminuria was also significantly elevated in diabetic SHR compared with control SHR (6.5 ± 0.6 vs. 0.9 ± 0.2 mg/day, P < 0.001). Induction of HO-1 with CoPP decreased albuminuria in control SHR (0.6 ± 0.1 mg/day) and in diabetic SHR (2.2 ± 0.6 mg/day, P < 0.05); however, albuminuria remained significantly higher in diabetic SHR treated with CoPP than control SHR.

**Glomerular integrity and apoptosis.** Under diabetic conditions, defects in the glomerular filtration barrier and podocyte injury can compromise overall renal health. Accordingly, we assessed glomerular albumin permeability as a marker of glomerular integrity and injury. Glomerular permeability to albumin was significantly greater in diabetic SHR compared with control SHR (Fig. 2B) (P < 0.05). Induction of HO-1 with CoPP had no effect in control SHR, as glomerular integrity was fully intact in both groups of nondiabetic rats. CoPP significantly reduced glomerular permeability to albumin in diabetic SHR; however, glomerular permeability remained significantly higher than in control nondiabetic SHR (Fig. 2B) (P < 0.05).

To begin to assess the molecular mechanisms by which CoPP impacts the integrity of the glomerular filtration barrier, we measured protein expression of nephrin and α3β1-integrin in isolated glomeruli from control and diabetic SHR with or
without CoPP treatment. As depicted in Fig. 3, A and B, protein expressions of glomerular α3- and β1-integrin were lower in diabetic SHR compared with control SHR, and CoPP restored expression to levels comparable to control SHR. Glomeruli isolated from diabetic SHR also had significantly lower nephrin expression than those of control SHR, and CoPP restored nephrin expression to control SHR values (Fig. 3C). Lower glomerular nephrin expression in diabetic SHR is further supported by the finding that urinary nephrin excretion was significantly greater in diabetic SHR compared with control SHR, and treatment of diabetic SHR with CoPP significantly reduced nephrin excretion levels (Fig. 3D).

Kidney sections were also assessed by TUNEL staining to evaluate the presence of apoptotic cells. Using propidium iodide as a nuclear counterstain (red), TUNEL staining revealed large numbers of apoptotic cells (green staining) in whole kidney sections from diabetic SHR compared with control SHR (Fig. 4A). Treatment of diabetic SHR with CoPP reduced the numbers of apoptotic cells compared with control SHR (Fig. 4A). In isolated glomeruli, caspase 3 was measured as a marker of apoptosis using immunofluorescence and Western blotting. Glomerular caspase 3 fluorescence intensity and protein expression levels were markedly elevated in diabetic SHR compared with control, and CoPP reduced caspase 3 fluorescence intensity and protein expression levels in diabetic SHR (Fig. 4, B and C) (P < 0.05). Together, these data support a role of HO-1 induction in improving glomerular integrity, in part, by reducing apoptosis in diabetic SHR.

Renal fibrosis. Diabetes in SHR was associated with renal fibrosis and increased extracellular matrix deposition throughout the kidney. In glomeruli, diabetic SHR had increased fibronectin and collagen IV staining compared with controls (brown staining, Fig. 5, A and B, respectively). The increase in collagen deposition was also associated with a significant elevation in urinary collagen excretion in diabetic SHR compared with control SHR (105 ± 14 vs. 18 ± 5 μg/day, respectively, P < 0.005). Induction of HO-1 with CoPP normalized glomerular fibronectin and collagen IV staining (Fig. 5, A and B, respectively) and significantly lowered urinary collagen excretion in diabetic SHR (30 ± 7 μg/day, P < 0.05) without a significant effect in control SHR (19 ± 3 μg/day).

Glomerular inflammation. We next assessed the ability of HO-1 to regulate macrophage infiltration and NF-κB activation in control and diabetic SHR. Diabetic SHR have significantly more infiltrating macrophages in glomeruli than control SHR, and CoPP treatment normalized macrophage infiltration (Fig. 5C). The glomerular phospho-IKK/IKK ratio and cortical P65-NF-κB activity were also significantly greater in diabetic SHR compared with controls (Fig. 6, A and B) (P < 0.05). CoPP treatment reduced the glomerular phospho-IKK/IKK ratio in diabetic SHR and abolished the elevation in cortical P65-NF-κB activity (Fig. 6, A and B) (P < 0.05). Similarly, glomerular sICAM-1 levels were significantly elevated in diabetic SHR compared with control SHR (P < 0.05), and CoPP attenuated the increase in sICAM-1 in diabetic SHR (Fig. 6C). Urinary MCP-1 excretion was also assessed as a maker of renal inflammation.

Fig. 3. Glomerular α3- and β1-integrin protein expression relative to β-actin (A and B) and glomerular nephrin staining and protein expression (C) in control and diabetic SHR with or without CoPP treatment (n = 5). Also shown is urinary nephrin excretion (D) in control and diabetic SHR with or without CoPP treatment (n = 8). *Significant difference from control SHR (P < 0.05). #Significant difference from diabetic SHR (P < 0.05).
inflammation. MCP-1 excretion was greater in diabetic SHR compared with control SHR (110 ± 15 vs. 33 ± 6 ng/day, P < 0.05) and was reduced by CoPP treatment in diabetic SHR (70 ± 22 ng/day, P < 0.05) as well as in control SHR (9 ± 2 ng/day, P < 0.05).

To verify the specificity of HO-1 induction on markers of inflammation, glomeruli from diabetic SHR were treated ex vivo with the HO inhibitor SnMP alone or in combination with CoPP. Glomerular ICAM-1 protein expression was greater in glomeruli from diabetic SHR compared with control SHR (P < 0.05), and ICAM-1 expression in diabetic SHR was normalized by CoPP treatment (Fig. 7A). SnMP alone had no effect on ICAM-1 expression in diabetic SHR; however, cotreatment of glomeruli with SnMP and CoPP blocked the CoPP-mediated decrease in ICAM-1 expression (Fig. 7A).
Glomerular oxidative stress. Oxidative stress contributes to the progression of renal inflammation and injury, and we have previously published that CoPP decreases renal cortical oxidative stress in control SHR (12). 8-OHdG excretion was measured in the current study as marker of oxidative stress in diabetic SHR. Urinary 8-OHdG excretion was significantly greater in diabetic SHR compared with control SHR (306 ± 28 vs. 177 ± 34 ng/day, \( P < 0.05 \)) and was reduced with CoPP treatment in diabetic SHR (135 ± 20 ng/day, \( P < 0.05 \)) as well as in control SHR (158 ± 34 ng/day).

Similarly, glomerular superoxide levels were assessed in diabetic SHR using DHE staining and lucigenin chemiluminescence (Fig. 7, B and C, respectively). Glomerular superoxide levels were elevated in diabetic vs. control SHR (\( P < 0.05 \)). Treatment of isolated glomeruli from diabetic SHR with CoPP significantly reduced superoxide production (Fig. 7, B and C, respectively) (\( P < 0.05 \)). SnMP treatment alone had no effect on superoxide production in isolated glomeruli from diabetic SHR; however, cotreatment of diabetic glomeruli with SnMP partially blocked the CoPP-mediated decrease in superoxide (Fig. 7C).

DISCUSSION

The identification of molecular mechanisms driving glomerular injury in diabetes is an active area of research due to the
current lack of effective treatment options. Our study provides evidence that HO-1 induction offers protection against glomerular injury in type 1 diabetic SHR, a rat model of DN in which hyperglycemia coexists with hypertension. The primary novel finding of our study was that induction of HO-1 promotes the maintenance of glomerular basement membrane function and the integrity of the filtration slit, thereby decreasing renal injury. Additional studies further determined that the molecular mechanisms contributing to HO-1-induced protection of glomeruli include inhibition of apoptosis, fibrosis, inflammation, and oxidative stress. We postulate that HO-1 induction prevents diabetes-mediated damage to the glomerular filtration barrier and decreases apoptosis via blocking the stimuli that trigger the inflammatory and oxidative stress responses, which could further mediate deterioration of renal function. Taken together, our findings suggest that induction of HO-1 in diabetes may be an effective treatment to limit the progression of DN.

Consistent with publications from our laboratory and others, HO-1 induction decreased blood pressure in diabetic SHR (6, 12, 16) and lowered blood glucose levels (30), both of which could limit the progression of diabetic renal injury. In the current study, induction of HO-1 with CoPP lowered blood pressure and blood glucose levels in both control and diabetic SHR; however, the percent decreases were comparable in both groups. Therefore, although reductions in both blood pressure and blood glucose levels may contribute to the renoprotective effects of HO-1 induction in diabetic SHR, they are unlikely to be the sole mechanisms responsible as the changes in both parameters are comparable in control and diabetic SHR. According to the study, the current study focused on additional mechanisms in diabetic SHR that would protect the kidney from diabetic insult. We have recently published that HO-1 induction provides protection against elevations in blood pressure and renal injury in control SHR (12). Consistent with this report and those from other laboratories (16, 34), in the current study HO-1 decreased proteinuria in both control and diabetic SHR; however, the effect was more pronounced in diabetic rats. Goodman et al. (15) recently demonstrated a marked increase in acute tubular damage in streptozotocin-induced HO-2 knockout mice and the induction of HO-1 attenuated this damage, further supporting a renoprotective role for HO-1 induction in diabetes (15).

There is increased interest and focus on the impact of diabetes on the glomerular filtration barrier leading to the progressive loss of renal function in DN (37–38). Indeed, we found that diabetic SHR had a marked increase in glomerular permeability to albumin, indicating severe loss of the barrier function of the glomerulus in our model, consistent with other studies (37–38). Importantly, induction of HO-1 mitigated the degree of glomerular damage. Renal fibrosis typically precedes the decline in renal function during diabetes (7), and we also found that CoPP treatment significantly reduced glomerular fibrosis. It should be noted that HO-1 induction more effectively lowered proteinuria than it improved glomerular integrity. However, it is difficult to assess the exact relationship between increases in the integrity of the glomerular barrier and decreases in proteinuria. Saleh et al. (37) has previously shown that decreases in protein excretion are greater than the decreases in albumin permeability in diabetic rats treated with an endothelin antagonist; therefore, even a small increase in the integrity of the glomerular filtration barrier may offer substantial benefits to overall renal health.

To further assess the mechanisms by which HO-1 regulates glomerular permeability, we assessed glomerular integrin expression and nephrin. Podocytes attach to the glomerular basement membrane through adhesion proteins, primarily α3β1-integrins, and interact with numerous transmembrane proteins, including nephrin, to form the slit diaphragm. Nephrin is a zipper-like protein that allows water and small molecules to
pass but is critical in maintaining the proper barrier function of the glomerular capillary wall to prevent loss of proteins (26, 40). Downregulation of α3β1-integrins and/or nephrin leading to the loss of podocyte attachment to the glomerular basement membrane has been identified as a leading cause of proteinuria in DN (2–3, 18). Saleh et al. (37, 38) have recently shown that streptozotocin-induced diabetic male Sprague-Dawley rats have reduced expression of nephrin and glomerular α3- and β1-integrins as well as increased nephrin excretion. In the current study, the reduction in proteinuria and glomerular permeability upon HO-1 induction in diabetic SHR was accompanied by marked restoration of the glomerular filtration barrier components. These data support our hypothesis that one mechanism by which the induction of HO-1 protects the kidney from damage during diabetes is by maintaining the glomerular filtration barrier. However, the question remains: how does HO-1 induction accomplish this?

Induction of HO-1 has been shown to block high glucose-stimulated apoptosis in cultured mouse podocytes as well as in podocytes from streptozotocin-induced diabetic male Sprague-Dawley rats (24). HO-1 upregulation by CoPP also attenuated diabetic injury in the pancreatic tissue of type 1 nonobese diabetic mice and increased expression of the anti-apoptotic proteins p-AKT and BcL-XL (24). In addition, induction of HO-1 with stannous chloride in diabetic SHR increased whole kidney expression of the antiapoptotic proteins Bcl-2 and AKT (6). Consistent with these reports, we found that induction of HO-1 decreased renal apoptosis as well as glomerular caspase 3 expression, suggesting that HO-1 protects glomeruli from diabetic-induced apoptosis.

Cell damage and apoptosis will elicit both an inflammatory and oxidative stress response, and HO-1 has been demonstrated to have anti-inflammatory and antioxidant properties. Moreover, immune cell infiltration, inflammatory cytokines, and the NF-κB signaling pathway have been shown to contribute to the progression of DN in patients and experimental animal models with type 1 diabetes (9, 13, 20, 41). The NF-κB subunits P50 and P65 are typically bound to the inhibitory protein 1κB (19). Once phosphorylated and activated, 1κB is degraded and NF-κB translocates into the nucleus to activate cytokine formation, including ICAM-1 and MCP-1, which are key players in monocyte/macrophage infiltration and leukocyte adhesion in animal models of DN (7, 10, 17, 19, 23, 33, 44, 50). Supporting a role for NF-κB activation and inflammation in DN, we found renal NF-κB activity, MCP-1 excretion, glomerular ICAM-1 expression, and macrophage infiltration all to be increased in diabetic SHR. Interestingly, HO-1 induction significantly reduced diabetes-induced increases in inflammatory markers. This is consistent with previous reports indicating that HO-1 induction produced a reduction of NF-κB-induced inflammation in the muscle from Goto-Kakizaki and streptozotocin-induced diabetic rats (28–29). Moreover, this effect of HO-1 on glomerular inflammatory markers was specific to exogenous HO-1 induction since an inhibitor of endogenous HO-1 did not alter glomerular ICAM-1 expression, yet it effectively blocked the CoPP-mediated inhibition of ICAM-1.

Oxidative stress is also involved in the progression of diabetic renal inflammation and injury (27, 29), and recent data from our laboratory have demonstrated that induction of HO-1 with CoPP reduces renal superoxide production in control SHR (12). Consistent with previous reports at the whole kidney level, we found indices of glomerular oxidative stress to be increased in diabetic SHR and treatment with CoPP normalized superoxide production in isolated glomeruli to control SHR levels. As noted above, this effect was specific to exogenous HO-1 induction since an inhibitor of endogenous HO-1 did not alter glomerular superoxide production expression, yet it partially blocked the CoPP-mediated inhibition of oxidative stress. Taken together, our data support the potential usefulness of HO-1 induction in protecting the kidney from diabetes-induced renal injury via both anti-inflammatory and antioxidant mechanisms. Future studies will determine the relative contribution of these mechanisms by comparing the effect of HO-1 induction with antioxidant and/or anti-inflammatory drugs.

Perspectives and significance. We postulate that diabetes-induced oxidative stress activates NF-κB inflammation, which triggers the activation of proinflammatory cytokines, perturbing glomerular injury and apoptosis. The study highlights that HO-1 induction protects the kidney from diabetes-induced glomerular injury via its antioxidant, antiapoptotic, and anti-inflammatory properties. Hypoxia-inducible factor-1α (HIF-1α) plays a key role in pathophysiological conditions as it affects cell survival and was found to be activated by cytokines and reactive oxygen species under normoxic conditions (48). HIF-1α could be regulated by NF-κB (48), and previous studies have shown that cobalt chloride (CoCl2) induced renal HIF-1α and reduced renal injury in a rat model of chronic kidney disease (11, 32). We have recent data suggesting that CoPP also increased renal HIF-1α protein expression in diabetic SHR compared with control SHR. Accordingly, induction of HIF-1α with CoPP could be an upstream signal of HO-1 activation. Future studies will determine the relationship between HIF-1α, HO-1, and angiogenesis in hypertensive diabetic animal models.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: A.A.E. provided conception and design of research; A.A.E., J.F., B.B., and M.A.S. performed experiments; A.A.E., J.F., B.B., and M.A.S. analyzed data; A.A.E. and J.C.S. interpreted results of experiments; A.A.E. prepared figures; A.A.E. drafted manuscript; A.A.E. and J.C.S. edited and revised manuscript; J.C.S. approved final version of manuscript.

REFERENCES


