Attenuation of diabetes-induced renal dysfunction by multiple exposures to low-dose radiation is associated with the suppression of systemic and renal inflammation

Chi Zhang,1,3 Yi Tan,2,3 Weiying Guo,4 Cai Li,3,5 Shunzi Ji,1 Xiaokun Li,1,3,6 and Lu Cai2,3,7

1School of Public Health of Jilin University, Changchun, China; 2Department of Pediatrics, University of Louisville, Louisville, Kentucky; 3Chinese-American Research Institute for Diabetic Complications, Wenzhou Medical College, Wenzhou; 4The First Hospital of Jilin University; 5School of Pharmacy of Jilin University, Changchun; 6Engineering Research Center of Bioreactor and Pharmaceutical Development, Ministry of Education, Jilin Agricultural University, Changchun, and Key Laboratory of Biotechnology Pharmaceutical Engineering, Wenzhou Medical College, Wenzhou, China; and 7Departments of Medicine and Radiation Oncology, University of Louisville, Louisville, Kentucky

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Zhang C, Tan Y, Guo W, Li C, Ji S, Li X, Cai L. Attenuation of diabetes-induced renal dysfunction by multiple exposures to low-dose radiation is associated with the suppression of systemic and renal inflammation. Am J Physiol Endocrinol Metab 297: E1366–E1377, 2009. First published September 29, 2009; doi:10.1152/ajpendo.00478.2009.—Renal protection against diabetes-induced pathogenic injuries by multiple exposures to low-dose radiation (LDR) was investigated to develop a novel approach to the prevention of renal disease for diabetic subjects. C57BL/6J mice were given multiple low-dose streptozotocin (STZ; 60 × 6 mg/kg) to produce a type 1 diabetes. Two weeks after diabetes onset, some of diabetic mice and age-matched nondiabetic mice were exposed whole body to 25 mGy X-rays every other day for 2, 4, 8, 12, and 16 wk. Diabetes caused a significant renal dysfunction, shown by time-dependent increase in urinary microalbumin (Malb) and decrease in urinary creatinine (Cre), and pathological changes, shown by significant increases in renal structural changes and PAS-positive staining. However, diabetes-induced renal dysfunction and pathological changes were significantly, albeit partially, attenuated by multiple exposures to LDR. Furthermore, LDR protection against diabetes-induced renal dysfunction and pathological changes was associated with a significant suppression of diabetes-increased systemic and renal inflammation, shown by significant increases in serum and renal TNFα, ICAM-1, IL-18, MCP-1, and PAI-1 contents. To further explore the mechanism by which LDR prevents diabetes-induced renal pathological changes, renal oxidative damage was examined by Western blotting and immunohistochemical staining for 3-nitrotyrosine and 4-hydroxynonenal. Significant increase in oxidative damage was observed in diabetic mice, but not diabetic mice, with LDR. Renal fibrosis, examined by Western blotting of connective tissue growth factor and Masson’s trichrome staining, was also evident in the kidneys of diabetic mice but not diabetic mice with LDR. These results suggest that multiple exposures to LDR significantly suppress diabetes-induced systemic and renal inflammatory response and renal oxidative damage, resulting in a prevention of the renal dysfunction and fibrosis.

diabetic nephropathy; renal oxidative damage; inflammatory factors; radio-adaptive response; radiation hormesis

DIABETIC NEPHROPATHY (DN) is one of the major microvascular complications of diabetic patients. Understanding the mechanisms responsible for the initiation and development of the diabetes-induced renal injury will be essential to develop an effectively preventive or therapeutic approach for DN (11). It has recently been appreciated that systemic and renal inflammation caused by hyperglycemia and hyperlipidemia play an important role in the renal oxidative damage that initiates the development of renal pathogenesis (14, 24, 26, 30).

Inflammatory mediators, including adipokines, chemokines, adhesion molecules, and cytokines, were all found to play critical roles in the setting of DN (30). A growing body of evidence indicates that recruitment of inflammatory cells from the circulation into renal tissue is a critical feature of DN. For instance, both tumor necrosis factor-α (TNFα) and interleukin-18 (IL-18) as proinflammatory cytokines were found to be involved in the pathogenesis of DN. It is known that ILs increase vascular endothelial permeability and are also involved in the proliferation of mesangial cells and matrix synthesis. As a multifunction player, TNFα contributes significantly to sodium retention and renal hypertrophy, since it promotes the local generation of reactive oxygen and/or nitrogen species (ROS and/or RNS), and alters the barrier function of the glomerular capillary wall, resulting in an enhanced albumin permeability (7, 8, 30). Infiltration of the activated T cells and monocytes is an important step to initiate renal tissue damage and eventually to cause a progressive loss of renal function (14, 24, 26, 30). Renal expression of chemokines and adhesive molecules such as monocyte chemoattractant protein-1 (MCP-1) and ICAM-1 provides the molecular mechanisms that lead to inflammatory cell migration in renal inflammatory pathogenesis (30).

To support the critical roles of these inflammatory mediators in the pathogenesis of DN, several experimental studies have shown the beneficial effects of different anti-inflammatory therapies in preventing or delaying the development of DN (15, 34, 37, 42). Therefore, various therapeutic approaches have been explored, but most if not all drugs need to be metabolized in the liver and excreted via the kidney, which will increase the renal working load. On the basis of this concern, ionizing radiation as an invasive approach has been investigated to prevent or treat chronic renal diseases (1, 35). Aunapuu et al. (1) have demonstrated the suppressive effect by single radiation at a moderate dose (3 Gy) on renal ablation-induced renal inflammation and pathological changes. This study suggests that radiation at the moderate doses may have the potential to modify the progression of chronic renal failure in rats.
To date, however, there was no study to apply chronic or multiple exposures to radiation at the moderate dose levels for renal therapy, which may be due to the renal damage under such conditions (35). We have investigated extensively the effect of low-dose radiation (LDR; ≪250 mGy low linear energy transfer (LET) radiation; see Ref. 25) in vitro and in vivo (4, 16, 17, 23). The previous studies showed that LDR has a stimulating effect on cell metabolism, cellular defenses such as immune response and antioxidant action, and cell proliferation without significant toxic effects (4, 16, 17, 23, 28, 33). Given that DN is predominantly related to diabetes-induced renal oxidative damage, we were interested in defining whether multiple exposures of diabetic animals to LDR can provide a preventive effect on diabetes-induced renal dysfunction and pathological changes. To investigate the possible mechanisms, the effect of LDR on diabetes-induced inflammatory response and oxidative damage was also investigated under nondiabetic and diabetic conditions.

**MATERIALS AND METHODS**

**Animals and treatments.** Male C57BL/6J mice, 10 wk old (18–22 g of body weight), obtained from Chinese Academy of Medical Science & Peking Union Medical College, were housed in the Experimental Animal Center of Jilin University at 22°C with a 12:12-h light-dark cycle and free access to rodent chow and tap water. Animals were kept under these conditions for ≳2 wk before being used for the experiments. All animal procedures were approved by the University Animal Care and Use Committee, which is certified by the Chinese Association of Accreditation of Laboratory Animal Care. The body weights of mice were measured every 3 days.

**Induction of diabetes in mice.** Animals were randomly divided into two groups, a streptozotocin (STZ)-treated diabetic group (Sigma Chemical, St. Louis, MO) and a nondiabetic group. Both groups of mice were fasted overnight before STZ was given by intraperitoneal injection at 60 mg/kg body wt daily for 6 consecutive days. The STZ was freshly dissolved in 0.05 M sodium citrate buffer (pH 4.5). Nondiabetic (control) mice received equivalent volume of the citrate buffer. Blood glucose level was determined using a Freestyle glucometer. Blood glucose was measured to avoid the potential influence of food intake amounts. We considered mice to be diabetic [i.e., diabetes mellitus (DM) group] when blood glucose was >12 mmol/L.

**LDR.** Mice from both the DM and age-matched control groups were randomly divided into two groups; one group was given multiple exposures to LDR (25 mGy X-rays) as control/LDR or DM/LDR, and another group was given sham exposure as control and DM. The mice from the DM/LDR and control/LDR groups were given X-ray exposure, using a Philip deep-therapy apparatus (Model X.S.S.205 FZ) with 200 kvp and 10 mA using 0.5 mm Cu and 1.0 Al as filter. The distance between X-ray source and animals was 212 cm with a dose rate of 12.5 mGy/min. LDR was given every other day at the same time. Eight mice from each group of the control and diabetic mice with and without multiple exposures to LDR were euthanized when exposure times reached 2, 4, 8, 12, or 16 wk.

**Measurements for renal function.** Mice were placed in individual metabolic cages for 24 h to collect urine samples under the condition of only access to tap water on the day before animals were euthanized. The urinary microalbumin (Malb) and creatinine (Cre) contents as parameters of renal function were detected using corresponding assay kits (Boster Biological Technology, Wuhan, China).

**ELISA.** The blood samples were collected from orbit and centrifuged (12,000 rpm, 10 min, 4°C) in heparinized centrifuge tubes, and the serum was stored at −70°C for ELISA. Serum ICAM-1, TNFα, IL-18, and MCP-1 levels were detected by using corresponding ELISA kits (R & D). Fifty microliters of the standard solutions and diluted samples were added into each well of the 96-well microtiter plates precoated with either ICAM-1 antibody, TNFα antibody, IL-18 antibody, or plasminogen activator inhibitor-1 (PAI-1) antibody. After incubation for 2 h, the wells were aspirated and washed five times with washing buffer. Then, a 100-μl aliquot of horseradish peroxidase-conjugated anti-mouse antibody of either ICAM-1, TNFα, IL-18, or PAI-1 was added, and the plates were incubated for 2 h at room temperature. After being washed three times with PBS, 100 μl of substrate solution was added to each well and incubated for 30 min at room temperature under dark conditions. By addition of stop solution, the reaction was stopped and the absorbance read at 450 nm by an EL800 Universal Microplate Reader (SPECTRA max PLU 384; Molecular Devices).

**RNA isolation and RT-PCR.** Mice were euthanized by cervical dislocation, and then the left kidney was harvested. After the renal capsule was removed, the kidney was divided into three parts for RT-PCR assay, Western blotting, and immunohistochemical staining. Tissues for RT-PCR and Western blotting were stored at −80°C. Tissues for immunohistochemical staining were fixed in 10% formaldehyde fluid.

Total RNA was isolated from kidney with RNAiso Reagent (TaKaRa). After phenol-chloroform extraction, isopropanol precipitation, and ethanol rinsing, the total RNA was dissolved in RNase-free water. Concentration and purity of RNA were determined by spectrophotometry (UV-26; Shimadzu) at wavelengths of 260 and 280 nm. Equivalent total RNA was reverse transcribed into cDNA, followed by amplification of the target genes by PCR. Primers were designed for ICAM-1, IL-18, MCP-1, PAI-1, and TNFα (Table 1). The ampli-

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MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1.

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fied PCR products were detected by agarose gel electrophoresis. The resulting bands were quantified with Lumi-Imager (Roche Diagnostics, Mannheim, Germany). The ratio of the intensity integral to the target PCR products to that of GAPDH was calculated and presented.

Western blotting. Tissue lysates were prepared in lysis buffer (1% Triton X-100, 150 mmol/l NaCl, 50 mmol/l Tris, pH 8.0, 1 mmol/l EDTA, 10 mg/l phenylmethylsulfonyl fluoride) using a homogenizer on ice. Then, the supernatants were collected after centrifugation at 12,000 rpm at 4°C for 20 min. Protein concentration was determined by the Bradford assay. Equal amounts (50 μg protein/lane) of protein and prestained molecular weight marker (Gibco-BRL, Gaithersburg, MD) were loaded onto 12% SDS-polyacrylamide gels in a minigel apparatus (Mini-Protean II; Bio-Rad). After being separated on an SDS-PAGE gel, proteins were transferred to nitrocellulose membranes (0.2 μm pore size; Bio-Rad). Nonspecific binding to the membrane was blocked overnight at room temperature with 5% nonfat milk in TBS buffer (20 mM Tris-HCl, 150 mM NaCl, and 0.1% Tween-20). The membranes were then incubated for 2 h at 37°C with primary antibodies in blocking buffer containing 8% milk. The primary antibodies included anti-mouse ICAM-1 polyclonal antibody, anti-mouse TNFα antibody, anti-mouse IL-18 antibody, anti-mouse MCP-1 antibody, and anti-mouse PAI-1 antibody (all from Sigma Chemical), rabbit anti-3-nitrotyrosine (3-NT) antibody (Upstate Biotechnology, Lake Placid, NY), and rabbit anti-4-hydroxynonenal (4-HNE) antibody (Alpha Diagnostic, San Antonio, TX). After washing with PBS three times, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody. The same membranes were probed with GAPDH as loading controls. The blots were developed with enhanced chemiluminescent reagent, exposed to film, and scanned using a Molecular Dynamics 300A Laser Densitometer.

Histological examination and immunohistochemical staining. The fixed kidney tissue was cut into 3-mm-thick blocks. The tissue blocks were embedded in paraffin and cut into 4-μm slices. After being deparaffinized using xylene and ethanol dilutions and rehydration, the sections were stained with hematoxylin and eosin (H & E), and periodic acid Schiff (PAS) and Masson’s trichrome staining were also performed, as described previously (32, 43).

For immunohistochemical staining, sections were blocked with Superblock buffer (Pierce, Rockford, IL) for 30 min. Sections then were incubated with the same primary antibodies as those used for Western blotting assays in 1:200 dilution overnight at 4°C. Following

Fig. 1. Effects of multiple exposures to low-dose radiation (LDR) on blood glucose levels, body weight, and renal function in nondiabetic and diabetic mice. A: the nondiabetic and diabetic mice were irradiated whole body with LDR (25 mGy X-rays) every other day for indicated times, and the blood glucose levels of mice for each group were measured at each time point. B: body weights were monitored weekly and presented at indicated time points. C and D: renal function was evaluated by measuring urinary microalbumin (Malb; C) and creatinine (Cre; D) with automatic biochemical-detecting equipment. Pre-LDR and w indicate that the samples were collected 1 day before and indicated times after the first exposure to LDR was given to both nondiabetic and diabetic mice. *P < 0.05 vs. diabetes mellitus (DM); †P < 0.05 vs. LDR; ‡P < 0.05 vs. control.
three times washing with PBS, these sections were incubated with biotin-labeled secondary antibody (Sigma) at room temperature for 1 h, followed by color development with diaminobenzidine for 2 min.

Statistical analysis. Data were collected from repeated experiments and are presented as means ± SD. Comparisons were performed by one-way ANOVA for the different groups, followed by unpaired Student’s t-test using statistical software SPSS 14.0. Differences were considered to be significant at \( P < 0.05 \).

RESULTS

Diabetic animal model and LDR effects. Hyperglycemic mice were induced by multiple low doses of STZ. One week after the last dose of STZ, whole blood glucose levels were examined under a condition of fasting for 6 h. Once hyperglycemia was diagnosed, diabetic mice and age-matched nondiabetic mice were divided into groups with and without exposure to LDR once every other day for 2, 4, 8, 12, and 16 wk. Figure 1A shows that multiple exposures of nondiabetic mice to LDR did not affect whole blood glucose levels. Whole blood glucose levels in the DM group increased significantly from 2 to 4 wk and then kept plateau levels (Fig. 1A). Blood glucose levels in the DM/LDR group were kept at plateau levels from 2 wk after the beginning of exposure to LDR and showed a statistical difference from those in the DM groups. Diabetes delayed the body weight gain, which is consistent with our previous studies (3, 4).

Fig. 2. Renal protection by multiple exposures to LDR against diabetes-induced histopathological changes. Renal pathology from different groups was examined at the indicated time points with light microscope by hematoxylin and eosin (A) and periodic acid Schiff (PAS; B) staining. Arrows indicate the positive sediment. C: semiquantitative analysis for the pathological changes was scored on the basis of deposited PAS-positive materials in glomeruli (0, +1, +2, +3, +4), as described previously (32, 43).
Multiple exposures to LDR did not affect diabetes and nondiabetic body weight gain (Fig. 1B).

Diabetes-induced renal dysfunction and pathogenic changes and LDR effects. It is known that when renal dysfunction occurs, urinary Malb levels increase and urinary Cre levels decrease. Figure 1 shows that diabetes induced a time-dependent renal dysfunction from 2 to 16 wk, shown by the increased urinary Malb (Fig. 1C) and decreased Cre (Fig. 1D) levels. In nondiabetic mice, urinary Malb was increased when given multiple exposures to LDR for 2 and 4 wk and reached plateau levels when exposed to LDR for longer periods (Fig. 1C). However, multiple exposures of diabetic mice to LDR for 4 wk or longer significantly reduced diabetes-caused urinary Malb levels (Fig. 1C). Multiple exposures to LDR very slightly decreased urinary Cre levels in nondiabetic mice but significantly ameliorated diabetes-decreased urinary Cre levels in diabetic mice (Fig. 1D). These results suggest that although multiple exposures to LDR slightly affect normal renal function, they significantly ameliorate diabetic renal dysfunction.

Renal histopathological examination by H & E (Fig. 2A) and PAS (Fig. 2B) staining disclosed that, compared with the control group, LDR-treated mice displayed a normal size of glomerulus without obvious Bowman’s capsule adhesion, mesangial cell proliferation, matrix expansion, and capillary collapse. Consistent with the functional findings, diabetic kidneys showed significantly increased size of glomerulus with obvious mesangial cell proliferation, mesangial matrix expansion, capillary wall thickness, and capillary collapse. The renal tubule dilation, epithelial cell degeneration, and interstitial wideness were also observed in the kidneys of diabetic mice. All of these pathological changes were more evident in the kidneys of diabetic mice at 8- to 16-wk time points (Fig. 2, A and B). Exposure to LDR partially but significantly prevented diabetes-induced renal pathological changes (Fig. 2, A–C).

Diabetes-induced systemic inflammation and LDR effects. To investigate the mechanisms by which multiple exposures to LDR attenuate diabetes-induced renal dysfunction and pathological changes, systemic levels of a few inflammatory mediators were measured by using ELISA assay. Figure 3 shows that multiple exposures of nondiabetic mice to LDR slightly increased serum TNF^\text{a}\alpha (Fig. 3A), IL-18 at 4 to 16 wk (Fig. 3B), and MCP-1 at all time points (Fig. 3C) but did not affect serum ICAM-1 (Fig. 3D). Diabetes significantly increased these inflammatory mediators starting from 2 or 4 wk.
until 16 wk. Multiple exposures of diabetic mice to LDR remarkably reduced these diabetes-caused inflammatory mediators (Fig. 3). These results suggest that diabetes significantly induces systemic inflammation, and LDR also induces a mild inflammatory effect in nondiabetic animals, but multiple exposures of diabetic mice to LDR remarkably prevent diabetes-induced systemic inflammation.

Diabetes-induced renal inflammation and LDR effects. To define the effects of LDR on diabetes-induced renal inflammation, renal expression of TNFα mRNA and protein was measured by RT-PCR (Fig. 4A) and Western blot (Fig. 4, B and C) assays. Diabetes induced remarkable increases in renal TNFα levels. By immunohistochemical staining, diabetes-increased TNFα was found to be expressed predominantly in glomeruli (Fig. 4D). Multiple exposures of nondiabetic mice to LDR did not significantly increase renal TNFα levels, except for mRNA at 8 wk and protein at the 8- and 12-wk time points, but did significantly attenuate diabetes-upregulated TNFα expression at both mRNA and protein levels (Fig. 4).

Renal ICAM-1 expression was also found to be significantly increased in diabetic groups at both mRNA (Fig. 5A) and protein (Fig. 5, B and C) levels. The localization of increased ICAM-1 was found predominantly in glomerulus and less in tubules (Fig. 5D). Although exposures to LDR slightly increased ICAM-1 expression at both mRNA and protein levels, it could prevent diabetes-induced ICAM-1 mRNA expression completely at 2 wk and partially at the 12- and 16-wk time points (Fig. 5A) and prevent diabetes-induced ICAM-1 protein expression almost completely at 2 and 4 wk and partially at the 8- to 16-wk time points (Fig. 5, A and B).

Consistent with the findings from serum study (Fig. 3), diabetes also significantly increased renal IL-18 and MCP-1 expressions at both mRNA (Fig. 6D) and protein levels (Fig. 6, B and C, for IL-18 and Fig. 6, E and F, for MCP-1) in a time-dependent manner. Multiple exposures to LDR that barely affected renal IL-18 expression and slightly increased MCP-1 in nondiabetic mice remarkably reduced diabetes-upregulated IL-18 and MCP-1 expressions at both mRNA and protein levels (Fig. 6).

Diabetes-increased renal oxidative damage and profibrotic response and LDR effect. It has been appreciated that pathogenic effects of inflammatory responses in the kidney are attributed mainly to its generation of oxidative stress and damage and stimulation of profibrotic response (7, 8, 11, 14, 22, 24, 26). Therefore, renal oxidative damage at exposure times of 8 wk was examined by immunohistochemical staining for oxidative and nitrosative damage markers: protein-nitration-related 3-NT and lipid peroxidation-related 4-HNE (Fig. 7A). There was no significant staining of 4-HNE and 3-NT in the kidneys of nondiabetic mice with and without exposure to LDR. How-

Fig. 4. Effects of multiple exposures to LDR on renal TNFα expression. Renal tissues from different groups at indicated times were collected to detect TNFα expression at mRNA level by RT-PCR (A), protein level by Western blotting (B and C), and cell type localization with immunohistochemical staining (D). Arrows indicate the positive staining. *P < 0.05 vs. DM; †P < 0.05 vs. LDR group; ‡P < 0.05 vs. control group. D/R, diabetes/LDR; D, diabetes; R, LDR; C, control.
ever, diabetes induced significant increases in 4-HNE and 3-NT staining predominantly in certain tubules and also less in the glomerulus, which could be almost completely prevented by multiple exposures to LDR (Fig. 7, A and B). The diabetic induction of nitrosative and oxidative damage was quantitatively confirmed by Western blotting of 3-NT (Fig. 7C) and 4-HNE (Fig. 7D).

In the next study, renal expression of PAI-1 was examined with RT-PCR and Western blot assay as well as immunohistochemical staining (Fig. 8) because PAI-1 acts not only as a proinflammatory factor but also as a profibrotic mediator. Multiple exposures to LDR slightly increased renal PAI-1 expression, starting from 2 wk and reaching plateau levels thereafter at mRNA (Fig. 8A) and protein (Fig. 8, B and C) levels. LDR-upregulated PAI-1 expression spreads in tubules and glomeruli (Fig. 8D). Diabetes significantly increased PAI-1 expression at both mRNA (Fig. 8A) and protein levels (Fig. 8, B and C) in a significantly time-dependent manner, and the upregulated PAI-1 expression was found in both tubules and glomeruli (Fig. 8D). Multiple exposures of diabetic mice to LDR also significantly attenuated diabetes-upregulated PAI-1 expression at early stages such as 2 and 4 wk and partially at the late stages such as 8, 12, and, in particular, 16 wk (Fig. 8, A and B).

Renal fibrosis was examined further by Western blotting of connective tissue growth factor (CTGF) expression (Fig. 9A) and Masson’s trichrome staining (Fig. 9B). Exposure of non-diabetic mice to LDR did not affect CTGF expression examined by Western blotting (Fig. 9A). Diabetes induced a significant increase in renal CTGF expression, which was completely prevented by exposures of diabetic mice to LDR (Fig. 9A). The staining showed that there was no significant glomerular fibrosis and sclerosis in all groups. However, in the kidneys of diabetic mice, the glomerular capsule adhesion, dilated mesangial matrix, and mild renal interstitial fibrosis (Fig. 9B) were shown by Masson’s trichrome staining, suggesting the existence of mild renal fibrosis since the postdiabetes times (the longest is 16 wk) were relatively short for the development of diabetic fibrosis. There was not significant renal fibrosis in the kidneys of diabetic mice exposed to LDR, suggesting that multiple exposures of diabetic mice to LDR also attenuated diabetes-caused mild renal fibrosis.

**DISCUSSION**

In the present study, we have for the first time provided evidence that multiple exposures to LDR such as 25 mGy of X-rays, although they caused slight renal inflammation, significantly attenuated diabetes-induced renal inflammation and oxidative damage, resulting in a significant prevention of renal dysfunction and pathological changes.
Inflammatory response has been considered as one of the major mechanisms by which diabetes causes renal oxidative injury, structural changes, and dysfunction (7, 8, 14, 24, 26, 30). Protective effects against DN by various anti-inflammatory therapies have recently been documented (15, 34, 37, 42). For example, Utimura et al. (34) have defined the effects of chronic anti-inflammatory therapy in experimental DM. They found that diabetic rats exhibited markedly glomerular hyperfiltration and hypertension and developed progressive albuminuria along with widespread glomerulosclerotic lesions associated with macrophage infiltration at 8 mo after diabetes onset. Treatment with mycophenolate mofetil (CellCept) as an immunosuppressive therapy and management of renal, cardiac, or hepatic transplant patients did not change rat blood pressure, glomerular dynamics, or blood glucose levels but significantly prevented albuminuria, glomerular macrophage infiltration, and glomerulosclerosis (34).

The relationships between inflammatory response and the development or progression of DN involve very complex processes. Diverse inflammatory molecules play significant roles in this scenario. These inflammatory molecules include adipokines, chemoattractant cytokines (chemokines), adhesion molecules, and proinflammatory cytokines (30). In the present study, we demonstrated the significant increases in the serum and renal proinflammatory cytokines [TNFα (Figs. 3 and 4) and IL-18 (Figs. 3 and 6A)] and chemoattractant cytokines [ICAM-1 (Figs. 3 and 5) and MCP-1 (Figs. 3 and 6B)]. Consequences of inflammatory response include the overgeneration of ROS and/or RNS that induce oxidative and nitrosative damage, as it was found in the present study that diabetes prevented albuminuria, glomerular macrophage infiltration, and glomerulosclerosis (34).

Fig. 6. Effects of multiple exposures to LDR on renal IL-18 and MCP-1 expression. Renal tissues from different groups at indicated times were collected to detect IL-18 and MCP-1 expressions at mRNA levels by RT-PCR (A and D) and protein levels by Western blotting (B and C for IL-18 and E and F for MCP-1). *P < 0.05 vs. DM; ′P < 0.05 vs. LDR group; ″P < 0.05 vs. control group.
increased renal accumulation of 3-NT and 4-HNE, detected by Western blotting and immunohistochemical staining (Fig. 7).

Both inflammatory response and oxidative damage further induce upregulation of profibrotic cytokines that initiate a gradual fibrotic response (9, 14, 24, 26, 39). To be consistent with this notion, we demonstrated the increased expression of PAI-1 mRNA and protein in the kidney of diabetic rats (Fig. 8).

PAI-1, as a major inhibitor of urokinase-type plasminogen activator and tissue plasminogen activator negatively regulates the formation and activity of plasmin, which can cleave most matrix proteins (22, 29). Increased PAI-1 expression leads to decreases in plasminogen activator activity and plasmin formation and, consequently, renal ECM accumulation. In addition, since PAI-1 also regulates the adhesion and migration of a variety of cells, the elevated PAI-1 may promote collagen deposition through stimulating migration of leukocytes and collagen-producing cells into the damaged tissues (22, 29).

Mice with PAI-1 deficiency were found to be resistant to diabetes-induced renal fibrosis (27). Consistent with these studies, the present study demonstrated that increased PAI-1 expression at mRNA and protein levels (Fig. 8) was accompanied by increased CTGF expression (Fig. 9A) and ECM accumulation, shown by increased PAS-positive materials (Fig. 2B) and Masson’s trichrome staining (Fig. 9B) in the kidneys of diabetic mice.

The novel finding of the present study is the significant protection by multiple exposures to LDR against diabetes-induced renal pathogenesis, along with a significant suppression of systemic and renal inflammation, and renal oxidative damage and fibrotic response. Anti-inflammatory effect of ionizing radiation has been noted for a long time at moderate doses (3–6 Gy) (1, 35). Although radiation at the moderate doses has suppressive effects on renal inflammatory responses (1, 35) and on ischemia-reperfusion-induced renal damage (20), its acute toxic effects and potentially late long-term effects are expected and a concern (20, 35).

Effects of LDR, referred mainly to those /11021250 mGy of low LET-ionizing radiation (see Refs. 2 and 25), are distinct from those of moderate- or high-level radiation (2, 4, 25). In general, LDR at dose ranges of <100 mGy was found to significantly

Fig. 7. Effects of multiple exposures to LDR on renal nitrosative and oxidative damage. After nondiabetic and diabetic mice with and without multiple exposures to LDR for 8 wk, renal nitrosative and oxidative damage was examined with immunohistochemical staining (A and B) and Western blot assays (C and D) for 3-nitrotyrosine (3-NT) and 4-hydroxynonenal (4-HNE). Arrows indicate the positive staining. aP < 0.05 vs. DM; bP < 0.05 vs. LDR group; cP < 0.05 vs. control group.
stimulate cellular metabolic activity (Akt signaling upregulation, DNA, and protein synthesis) and increase cellular antioxidant action (increased antioxidant contents and activities) and cell proliferation, which makes LDR-irradiated cells or tissues more resistant to subsequent radiation- or chemical-induced damage (2, 6, 19, 25, 28).

Effects of LDR on normal cells and tissues have been documented as both pro- and anti-inflammatory, depending on the experimental conditions (12, 13, 18, 31). In the present study, we also found the induction of mild systemic and renal inflammation and even mild renal pathological changes by multiple exposures to LDR in nondiabetic mice (Figs. 1–8), further supporting the aforementioned previous studies. However, multiple exposures of diabetic mice to LDR could significantly attenuate diabetes-induced systemic and renal inflammation, resulting in a significant prevention of the development of diabetic renal pathogenesis. One explanation for both pro- and anti-inflammatory effects of LDR may be that LDR-initiated mild inflammation along with mild tissue damage turns on the systemic and cellular defense mechanisms, including upregulation of cellular metabolic activities, antioxidant capacities, and antiapoptotic signalings. These defense mechanisms make the tissues more resistant to diabetes-induced renal inflammation and associated oxidative damage. Previous studies have reported the induction of antioxidants in different tissue by exposure to LDR (10, 21, 28, 36, 40, 41). We also demonstrated the preventive effect on diabetes-induced oxidative damage by multiple exposures to LDR (Fig. 7).

The stimulatory effect of LDR on tissue and cell proliferation may also account for, at least in part, the protection against diabetes-induced renal inflammation and oxidative damage. Figure 1A clearly shows that, before diabetic mice were exposed to LDR, fasting blood glucose levels were the same between diabetic mice with and without exposure to LDR, but the fasting blood glucose levels are consistently lower in diabetic mice with LDR exposure than those in diabetic mice without LDR exposure. Whether this difference is due to the stimulation of residual β-cell proliferation in pancreas will be further explored in future studies.

In summary, we provide for the first time the evidence that multiple exposures to LDR (25 mGy X-rays) can significantly prevent diabetes-induced systemic and renal inflammation, which was accompanied with a significant prevention of diabetes-induced renal pathogenic effects, including renal oxidative damage and structural and functional changes. Although the dose used in the present study is a publicly acceptable dose that can be attributed by a whole body computer tomography diagnostic examination, whether there is a potential long-term adverse effect remains to be defined in future studies. However, the balance between the potentially therapeutic benefits and the long-term potentially mild toxic effects should be considered for diabetic patients who may develop severe DN

Fig. 8. Effects of multiple exposures to LDR on renal plasminogen activator inhibitor-1 (PAI-1) expression. Renal tissues from different groups at indicated times were collected to detect PAI-1 expression at mRNA level by RT-PCR (A), protein level by Western blotting (B and C), and cell type localization with immunohistochemical staining (D). Arrows indicate the positive staining. *P < 0.05 vs. DM; †P < 0.05 vs. LDR group; ‡P < 0.05 vs. control group.
earlier than the occurrence of the late adverse effects of LDR. Furthermore, given that whole body exposure to LDR is so easy and convenient to perform, this may provide a novel therapeutic approach for diabetic patients in the near future.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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