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8-Oxo-2′-deoxyguanosine ameliorates features of metabolic syndrome in obese mice

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ABSTRACT

Metabolic syndrome describes a group of clinical features that together increase the incidence of coronary artery disease, stroke and type 2 diabetes. Insulin resistance is a major risk factor for developing metabolic syndrome. A chronic state of inflammation accompanies the accumulation of surplus lipids in adipose and liver tissue, frequently involved in insulin resistance. 8-Oxo-2′-deoxyguanosine (8-Oxo-dG) is a potent anti-inflammatory agent that inactivates both Rac1 and Rac2 which are critical to initiating the inflammatory responses in various cell types, including macrophages. In this study, we explored whether 8-Oxo-dG suppressed a series of systemic inflammatory cascades, resulting in the amelioration of typical features of metabolic syndrome in obese mice. The results demonstrate that 8-Oxo-dG effectively improved hyperglycemia, dyslipidemia and fatty liver changes in obese mice. The level of biochemical markers indicative of systemic inflammation were reduced in 8-Oxo-dG treated mice, whereas serum levels of adiponectin, a crucial factor associated with improved metabolic syndrome, were enhanced. Our results demonstrate that 8-Oxo-dG effectively disrupts the pathogenesis of insulin resistance and obesity-associated metabolic syndrome.

1. Introduction

Obesity is a metabolic disease of pandemic proportions [1]. The most common type of obesity is polygenic, resulting from long-term energy excess stored in adipose tissue [2]. In obesity, some yet undefined mechanisms lead to insulin resistance and increase the risk of cardiovascular or cerebrovascular disease [3]. Metabolic syndrome also increases the risk of cardiovascular disease and type 2 diabetes mellitus (T2DM) [4]. Individuals with metabolic syndrome have a threefold greater risk of suffering a cardiovascular or cerebrovascular event and fivefold greater risk of developing T2DM when compared to subjects without metabolic syndrome [5].

Adipose tissue is a heterogeneous mix of adipocytes, periadipocytes, immune cells and endothelium. Pro-inflammatory cytokines are upregulated in plasma from obese subjetsm which links obesity with inflammation [6]. Several micro-environmental factors such as hypoxia, induce adipocyte enlargement in obese subjects and trigger overexpression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) or interleukin-6 (IL-6) [7,8]. Localized inflammation in adipose tissue gradually progresses to a systemic inflammation, which is associated with the development of obesity-related consequences [9].

Many of the typical pro-inflammatory stimuli activate c-Jun N-terminal kinase (JNK) and inhibitor of κB kinase beta (IKKβ pathways, which play important roles in inflammation-associated insulin resistance [10]. JNK induces insulin resistance through the phosphorylation of serine residues in insulin receptor substrate-1 (IRS-1) [11,12]. Insulin receptor signaling transmitted through a tyrosine kinase pathway, is suppressed by counter-regulatory serine/threonine phosphorylation events [13].

In a previous study, we showed that 8-Oxo-2′ deoxyguanosine (8-Oxo-dG) inactivates small GTP-binding proteins such as Rac1 or Rac2 [14,15], resulting in the inhibition of Rac-linked functions including reactive oxygen species (ROS) production, phagocytosis, chemotaxis and nitric oxide (NO) production [15,16]. Consistent

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with these findings, 8-Oxo-dG exhibited anti-inflammatory properties against lung inflammation induced by lipopolysaccharide (LPS) or ovalbumin [17]. In this study, we demonstrated that 8-Oxo-dG potently ameliorated the features of metabolic syndrome, including hyperglycemia, dyslipidemia and fatty liver changes, in obese mice by down-regulating the expression of pro-inflammatory cytokines such as TNF-α, IL-6 and matrix metalloproteinase-9 (MMP-9). In contrast, 8-Oxo-dG significantly enhanced the plasma level of adiponectin. These results provide rationale for the development of 8-Oxo-dG as a potent candidate to improve obesity-associated insulin resistance and metabolic syndrome.

2. Materials and methods

2.1. Animals and treatment

Six-week-old male C57BL/6, KKAy, and db/db mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). KKAy mice are the result of a cross between glucose-intolerant black KK female mice and yellow male obese Ay mice, and are known to serve as excellent models of obese insulin resistant T2DM [18]. Non-diabetic C57BL/6 mice were employed generally as controls for KKAy mice [19]. The metabolic abnormalities demonstrated by the db/db mouse, which are obese by 3–4 weeks of age, are due to a defect in leptin signaling resulting from a point mutation in the gene for the leptin receptor [20].

All animals were maintained under specific pathogen-free conditions and all experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Medicine, Seoul National University (Seoul, Korea). 8-Oxo-dG was purchased from Barry & Associates, Inc. (Dexter, MO, USA) and was administrated orally (60 mg/kg/day) by oro-gastric tube.

2.2. Fasting blood glucose determination

Blood samples were collected in the fasting state from the retro-orbital sinus once every 2 weeks. Approximately 50 μl of a fresh blood samples were placed on duplicate test strips and the glucose content was read in a validated one touch basic glucose measurement system (Lifescan Inc., Milpitas, CA, USA).

2.3. Other biological assays

Plasma insulin was measured using an ELISA kit (Linco, St. Charles, MO, USA) with rat insulin as a standard. The percent Hemoglobin A1c (HbA1c) was measured with a hemoglobin A1c kit (BioSystems S.A., Barcelona, Spain). Serum triglyceride (TG), low- density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol were measured using the TG, LDL, and HDL cholesterol kits (Daiichi Pure chemicals Co., Ltd, Tokyo, Japan). Serum adiponectin was analyzed with an adiponectin RIA kit (Linco, St. Charles, MO, USA) and measured using a gamma counter COBRA-II (Packard Co. Ltd., USA). Serum TNF-α, IL-6 and MMP-9 levels were measured using the Quantikine Mouse TNF-a, IL-6 and MMP-9 kit (R&D System Inc., Minneapolis, MN, USA), respectively and the absorbance was detected using a V-MAX 220 VAC ELISA reader (Molecular Devices, Sunnyvale, CA, USA).

2.4. Measurement of malondialdehyde (MDA) levels in liver

The hepatic MDA levels in each group of mice were measured using a lipid peroxidation colorimetric/fluorimetric assay kit (BioVision Inc., Milpitas, CA, USA) according to the manufacturer's instructions. In brief, ~10 mg pieces of liver were isolated and homogenized in the MDA lysis buffer. A thiobarbituric acid (TBA) solution was added to each vial containing standard or sample and Incubated at 95 °C for 60 min. The colorimetric analyses of MDA-TBA mixtures were performed using a V-MAX 220 VAC ELISA reader (Molecular Devices).

2.5. Hematoxylin and eosin (H&E) staining

Small pieces of liver specimens from each group of mice were fixed by incubation in 10% neutralized formalin for 24 h. Fixed tissues were embedded in paraffin and cut to a thickness of 4 μm. Paraffin-embedded slides were hydrated with ethanol and distilled water, and stained with H&E (Sigma–Aldrich), as described previously [21]. Image acquisition and processing were performed using a Leica microscope and the Leica Application Suite (Leica Microsystems, Buffalo Grove, IL, USA).

2.6. Measurement of Rac1 activity

Rac activation assays were conducted using Rac activation kits (Upstate Biotechnology, Lake Placid, NY, USA) as described previously [16]. Briefly, tissue lysates of white adipose tissue (WAT) from each group of mice were affinity-precipitated with 5 μg of GST-PAK-PBD (a fusion protein of glutathione *S*-transferase and the p21-binding domain of the p21-activated protein kinase 1) conjugated to glutathione-agarose beads. After elution with sodium dodecyl sulfate (SDS) sample buffer, 10% of whole protein lysate volume for input samples and eluted proteins were subjected to SDS–polyacrylamaide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes. The membranes were then probed overnight at 4 °C with the anti-Rac1 antibody (Upstate Biotechnology), followed by further incubation in horseradish peroxidase (HRP)-conjugated secondary antibody. The protein bands were visualized using SuperSignal West Femto Chemiluminescent Substrate (Thermo Fisher Scientific).

2.7. Statistical analysis

All values are expressed as means ± standard error of the mean (SEM). Two-tailed Student's *t*-tests were conducted using GraphPad Prism software, version 5.01 (GraphPad Software, LaJolla, CA, USA).

3. Results

3.1. 8-Oxo-dG ameliorates obesity-associated hyperglycemia and insulin resistance

Obese mice models such as KKAy or db/db usually develop metabolic disturbances, characterized by insulin resistance, hyperglycemia, dyslipidemia, and steatosis [18,20]. Moreover, obesity-associated systemic inflammation plays a crucial role in the development of metabolic disease [3,11].

Thus, we determined whether 8-Oxo-dG, as a potent anti-inflammatory agent, could improve various features of metabolic syndrome in obese mice models. Improvement in weight gain or excess food consumption in KKAy and the db/db mice was not detected in 8-Oxo-dG treated mice (Supplementary Fig. 1A and B). The plasma levels of fasting glucose were increased for both KKAy and the db/db mice at 8 weeks and were further elevated at 10 weeks. Ameliorations of impaired fasting glucose were detected from 4 or 6 weeks after oral treatment of 8-Oxo-dG (60 mg/kg/day) in KKAy or db/db mice, respectively (Fig. 1A). Consistent with these results, serum HbA1c, which reflects the average blood sugar level over the previous 3 months, was also elevated in both KKAy and

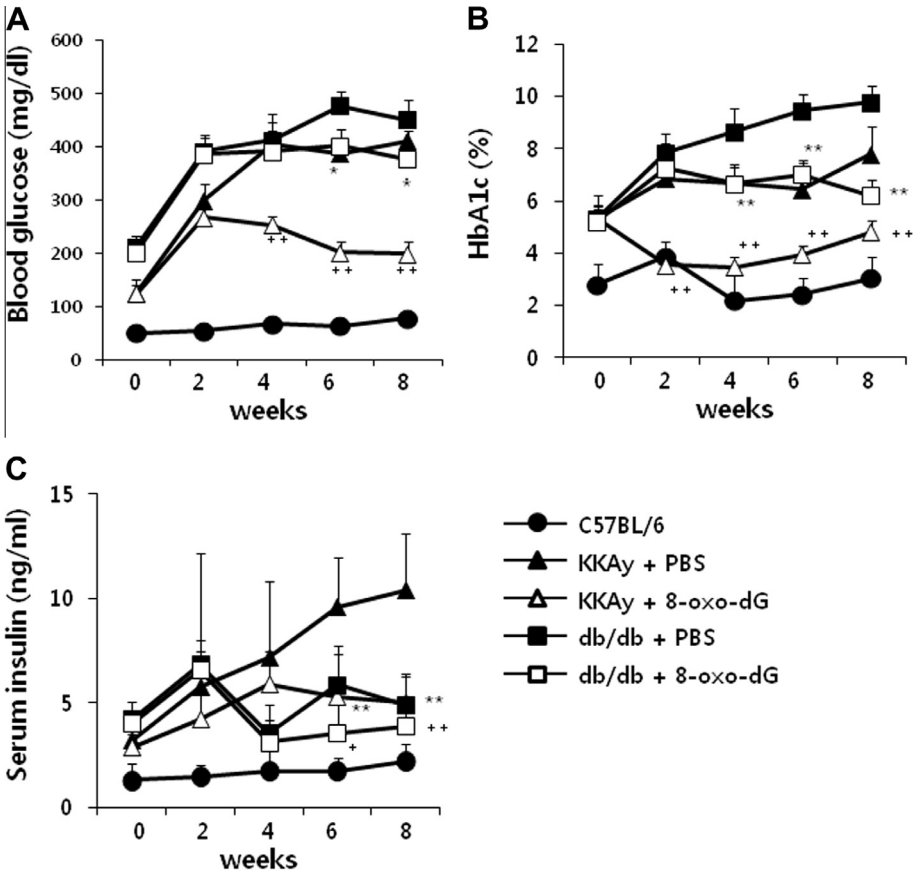

Fig. 1. The effect of 8-Oxo-dG on hyperglycemia and hyperinsulinemia in obese mice. The serum levels of fasting glucose (A), hemoglobin A1C (HbA1C) (B) or insulin (C) was first measured at 6 weeks in C57BL/6, KKAy and db/db mice. PBS or 60 mg/kg of 8-Oxo-2-deoxyguanosine (8-Oxo-dG) dissolved in sterile PBS was administered by oro-gastric tube once per day to KKAy and db/db mice. Thereafter, fasting glucose, HbA1C or insulin was repetitively measured every 2 weeks until 8 weeks after the first treatment. Results are presented as means ± SEM (n = 6/group). p values were calculated by two-tailed Student's t-test. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs db/db with PBS; $^{+}p < 0.05$ and $^{++}p < 0.01$ vs KKAy with PBS.

the db/db mice, and notably decreased in 8-Oxo-dG treated group (Fig. 1B). Moreover, plasma levels of fasting insulin, which represents insulin insensitivity, was significantly increased in obese mice models, and was reversed when mice were treated with 8-Oxo-dG (Fig. 1C). These findings collectively affirm that 8-Oxo-dG effectively ameliorates the hyperglycemia and insulin sensitivity increase in obese mice, without affecting weight gain.

3.2. 8-Oxo-dG improves dyslipidemia and fatty liver changes in obese mice

The deteriorations of lipid metabolism, including increased serum levels of TG or LDL cholesterol and decreased plasma HDL cholesterol, are the typical features of metabolic syndrome and the major risk factors for developing cardiovascular disease [22]. Moreover, patients with dyslipidemia frequently suffer from steatosis, a non-alcoholic fatty liver disease [23]. The serum levels of total cholesterol, TG and LDL cholesterol were notably increased, while that of HDL cholesterol was unchanged, in KKAy mice when compared to C57BL/6 mice (Fig. 2A). The serum level of total cholesterol did not change between PBS and 8-Oxo-dG treated KKAy mice (Fig. 2A). However, 8-Oxo-dG effectively lowered the serum level of TG and LDL cholesterol while significantly enhancing serum HDL cholesterol (Fig. 2A). The fatty liver changes that developed in KKAy and db/db mice, pathologically defined as an abnormal lipid disposition within hepatocytes, was dramatically reversed in the 8-Oxo-dG treated group when compared to the PBS control mice (Fig. 2B). The hepatic MDA level, which reflects hepatocyte lipid peroxidation, is typically increased in steatosis [24]. In KKAy and db/db mice, the hepatic MDA levels were enhanced, an increase that was potently reversed in the 8-Oxo-dG treated group (Fig. 2C). These results assert that 8-Oxo-dG effectively ameliorated lipid metabolism disturbances in obese mice.

3.3. 8-Oxo-dG inhibits obesity-associated systemic inflammation and enhances the serum adiponectin

Increased serum levels of TNF-α, IL-6 and/or MMP-9 are hallmarks of systemic inflammation, and strongly associated with obesity-associated metabolic diseases [4,11]. In previous studies, 8-Oxo-dG potently reduced the expression of various pro-inflammatory cytokines triggered by treatment with LPS or ovalbumin [16,17]. We found that the serum levels of TNF-α, IL-6 and MMP-9 were all increased in KKAy mice when compared to C57BL/6 controls (Fig. 3A–C). 8-Oxo-dG notably lowered serum TNF-α and IL-6 levels in KKAy mice, but had no effect in C57BL/6 control mice (Fig. 3A and B). However, 8-Oxo-dG significantly reduced the serum level of MMP-9 in both control and obese mice (Fig. 3C). Furthermore, the serum level of adiponectin was decreased in KKAy mice when compared to C57BL/6 control group (Fig. 3D) and 8-Oxo-dG significantly elevated the serum adiponectin levels of control and KKAY and db/db obese models (Fig. 3D and E).

3.4. 8-Oxo-dG suppresses Rac1 activity in white adipose tissue from obese mice

The anti-inflammatory properties of 8-Oxo-dG are attributable primarily to inhibition of Racc1/2 [16,17]. The Rho GTPase Rac

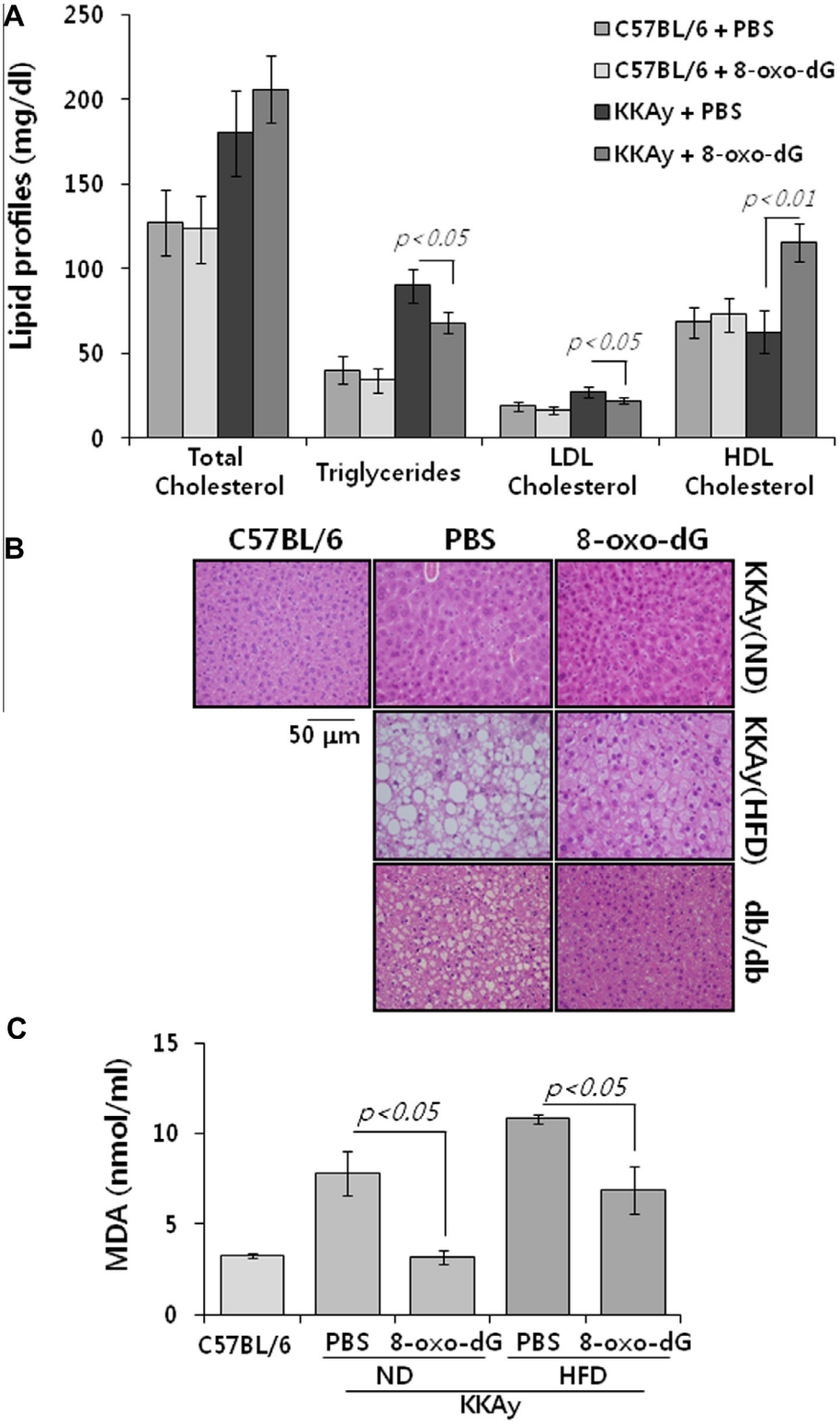

Fig. 2. The effect of 8-Oxo-dG on dyslipidemia and steatosis in obese mice. (A) The serum levels of triglyceride (TG), LDL-cholesterol and HDL-cholesterol was measured after 8 weeks of PBS or 8-Oxo-dG treated C57BL/6, KKAy or db/db mice. Results are presented as means ± SEM (n = 6/group). p values were calculated by two-tailed Student's t-test. (B) Liver tissues from C57BL/6, KKAy or db/db mice treated with PBS or 8-Oxo-dG for 8 weeks were isolated, fixed and embedded in paraffin for H&E staining. Data are representative in six mice per group. (C) Liver tissues (10 mg) from C57BL/6, KKAy or db/db mice treated with PBS or 8-Oxo-dG for 8 weeks were isolated and homogenized in the MDA lysis buffer to measure lipid peroxidation. Data are presented as the means ± SEM (n = 6/group). p values were calculated by two-tailed Student's t-test.

plays a key role in a variety of inflammatory responses by stimulating ROS production and regulating NF-κB activity [25]. Rac1 is generally bound GTP when it is activated; thus, an assay of GTP-bound Rac1 was performed to analyze Rac1 activity in WAT from each group of mice (Fig. 4A and B) [16]. Rac1 activity was notably enhanced in KKAy mice when compared to C57BL/6 controls

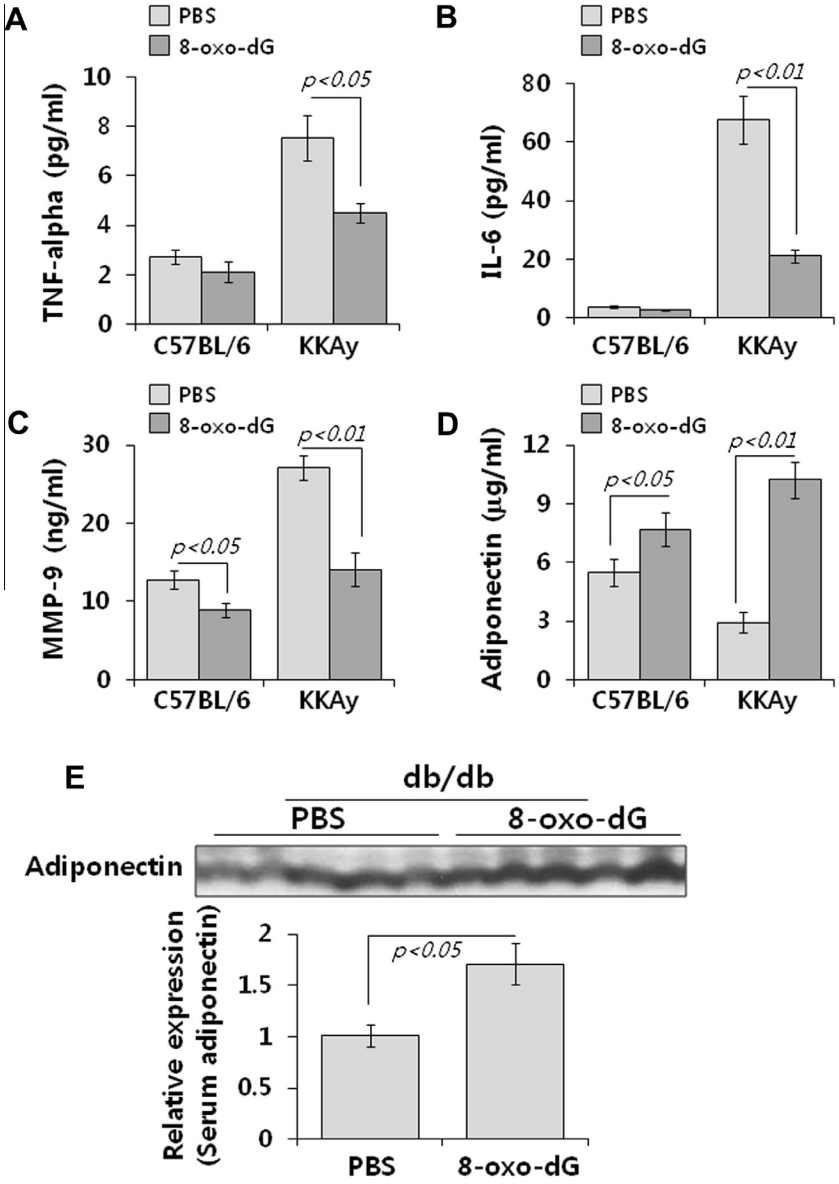

Fig. 3. 8-Oxo-dG suppresses the serum markers of systemic inflammation and enhances plasma adiponectin in obese mice. The serum levels of TNF-α (A), IL-6 (B), MMP-9 (C) and adiponectin (D) were measured by ELIZA in C57BL/6 or KKAy mice treated with PBS or 8-Oxo-dG for 8 weeks. Data are presented as means ± SEM (n = 6/group). p values were calculated by two-tailed Student's t-test. (E) Serum adiponectin levels were measured by immunoblot. In brief, isolated serum from db/db mice treated with PBS or 8-Oxo-dG for 8 weeks was diluted in SDS sample buffer and boiled for 5 min prior to SDS–PAGE. Immunoblots were probed with an anti-mouse adiponectin antibody. Data are representative of three independent experiments (upper pannel). The band densities are shown as a bar graph (lower pannel) and presented as means ± SEM (n = 6/group). p values were calculated by two-tailed Student's t-test.

(Fig. 4A) and 8-Oxo-dG significantly suppressed Rac1 activity in control and KKAy and db/db obese models when compared to PBS-treated animals (Fig. 4A and B).

4. Discussion

In this study, we demonstrated that 8-Oxo-dG effectively ameliorated typical features of metabolic syndrome including hyperglycemia (Fig. 1), dyslipidemia, and steatosis (Fig. 2) in obese mouse models. We also demonstrated anti-inflammatory activity of 8-Oxo-dG against obesity-associated systemic inflammation (Fig. 3), which was attributed to inhibition of Rac1 in WAT (Fig. 4).

8-Oxo-dG, a nucleoside with an oxidatively modified base, is generated upon DNA damage generated by chemicals, ultraviolet light or irradiation [26]. 7,8-Dihydro-8-oxoguanine (8-Oxo-Gua) in DNA has mutagenic properties because it induces a GC to TA transversion during replication [27]. For that reason, 8-Oxo-dG is normally eliminated through the base excision repair mechanism initiated by oxoguanine DNA glycosylase (OGG1) [28,29]. However, we reported previously that exogenous 8-oxo-dG was not generally incorporated into DNA, while cytosolic 8-Oxo-G could promote mutagenesis [30].

TNF-α is a pro-inflammatory cytokine that influences lipid metabolism and insulin signaling in adipose tissue [4]. TNF-α also triggers insulin resistance by inhibiting the insulin receptor substrate 1 (IRS1) signaling pathway [31]. The primary source of circulating IL-6 is macrophages that infiltrate WAT. IL-6 has an important role in the regulation of whole-body energy homeostasis and inflammation [4]. A study that investigated the plasma level of MMP-9 in obese subjects showed that circulating MMP-9 was significantly elevated with insulin resistance [32]. Moreover, induction of MMP-9 in insulin resistance potentially contributed to increased cardiovascular morbidity and mortality in T2DM [33].

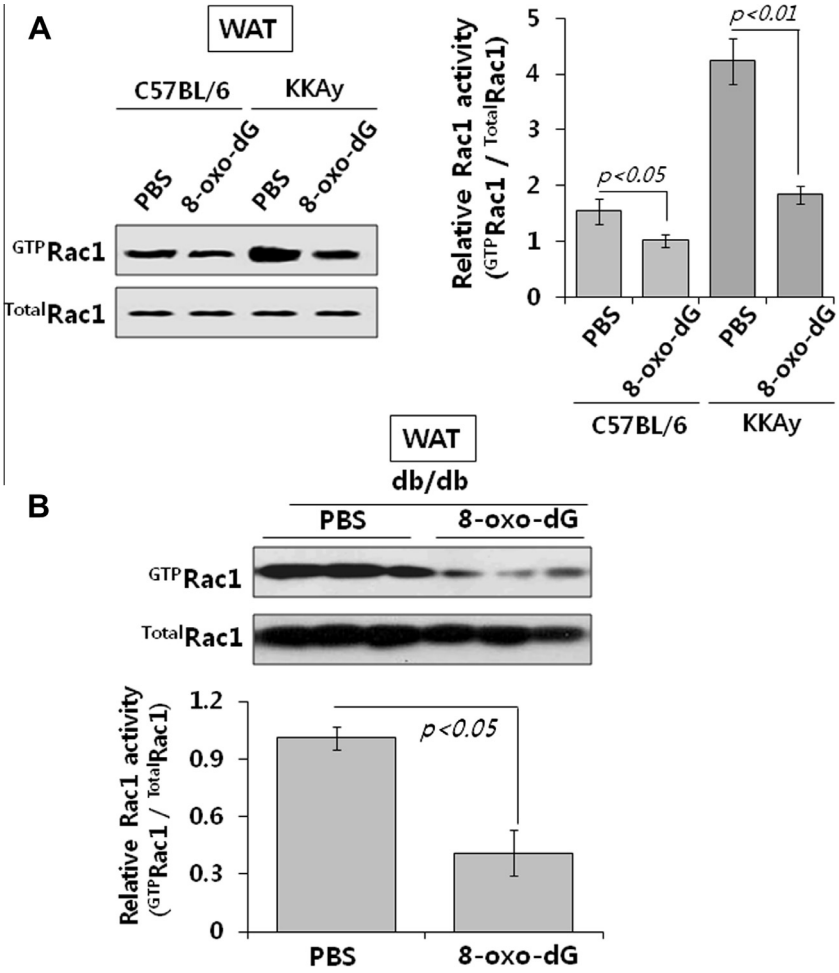

Fig. 4. 8-Oxo-dG suppresses Rac1 activity in white adipose tissue (WAT) from obese mice. (A) Rac1 activity was analyzed in WAT from C57BL/6 or KKAy mice treated with PBS or 8-Oxo-dG for 8 weeks. Data are representative of three independent experiments (n = 6/group) (left). Relative densities of active Rac1 (^{GTP}Rac1) divided by total Rac1 is presented as a bar graph (right). p values were calculated by two-tailed Student's t-test. (B) Rac1 activities in WAT from db/db mice treated with PBS or 8-Oxo-dG. Data are representative of three independent experiments (n = 6/group) (upper pannel). Relative Rac1 activities are shown as a bar graph (lower pannel). Data were presented as means ± SEM (n = 6/group). p values were calculated by two-tailed Student's t-test.

Systemic inflammation induced by pro-inflammatory cytokines reduced levels of anti-inflammatory cytokines such as adiponectin [34]. Adiponectin regulates lipid and glucose metabolism, increases insulin sensitivity and protects against the chronic inflammation [35]. Furthermore, decreased circulating adiponectin has been associated with insulin resistance, hyperinsulinemia and the development of T2DM [36].

Rac GTpase is essential to activation of NAPDH oxidase (NOX2), which has been identified as a key mediator of inflammation [37]. NOX2 also plays a fundamental role in releasing pro-inflammatory cytokines including IL-1β, TNF-α and IL-6 in the adipose tissue [38]. We previously discovered that 8-Oxo-dG inactivated Rac, and consequently, attenuated the NOX2 activity. 8-Oxo-dG also diminished the expression of pro-inflammatory cytokines, including TNF-α, IL-6 and IL-8, during LPS-induced inflammation [16]. In the present study, we consistently observed that 8-Oxo-dG potently inhibited Rac1 activity in WAT from obese mouse models (Fig. 4), which led to the down-regulation of pro-inflammatory cytokines such as TNF-α, IL-6 and MMP-9 (Fig. 3). The enhanced circulating adiponectin in the 8-Oxo-dG treated group may be attributable to decreased serum levels of TNF-α and IL-6. Decreased systemic inflammation, together with increased circulating adiponectin, in 8-Oxo-dG-treated mice could lead to enhanced insulin sensitivity and amelioration of the features of obesity-associated metabolic syndrome (Figs. 1 and 2).

In conclusion, our results demonstrate that 8-Oxo-dG ameliorated the typical features of metabolic syndrome through the suppression of obesity-associated systemic inflammation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.12.018.

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