Chinese Journal of Physiology 55(3): 192-201, 2012
DOI: 10.4077/CJP.2012.BAA012

Effect of Resveratrol on Oxidative and Inflammatory Stress in Liver and Spleen of Streptozotocin-Induced Type 1 Diabetic Rats

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Abstract

It has been well known that both oxidative stress and inflammatory activity play crucial roles in the pathogenesis of type 1 diabetes mellitus (T1DM). Resveratrol (RSV), a naturally occurring polyphenol found in grapes and red wine, has recently been shown to exert potent anti-diabetic, anti-oxidative and anti-inflammatory actions. In the present study, we investigated the effect of RSV on oxidative stress and inflammatory response in the liver and spleen of streptozotocin (STZ)-induced type 1 diabetic animal models. Male Long-Evans rats were injected with 65 mg/kg STZ to induce diabetes for 2 weeks, and subsequently administrated with the dosage of 0.1 or 1 mg/kg/day RSV for 7 consecutive days. Hepatic and splenic tissues were dissected for evaluation of oxidative and inflammatory stress. Oxidative stress was assessed by quantification of oxidative indicators including superoxide anion content, lipid and protein oxidative products, as well as manganese superoxide dismutase (Mn-SOD) and nitro-tyrosine protein expression levels. Inflammatory stress was evaluated by the levels of nuclear factor κB (NF-κB) and the proinflammatory cytokine tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β) and IL-6. The experimental results indicated that RSV significantly decreased oxidative stress (superoxide anion content, protein carbonyl level and Mn-SOD expression) in both tissues and hepatic inflammation (NF-κB and IL-1β), but implicated proinflammatory potential of RSV in diabetic spleen (TNF-α and IL-6). The results of this study suggest that RSV may serve as a potent antioxidant, but RSV possesses a proinflammatory potential in certain circumstances in diabetes.

Key Words: type 1 diabetes mellitus, liver, spleen, oxidative stress, inflammation, resveratrol

Introduction

Type 1 diabetes mellitus (T1DM) is a well known endocrine disorder derived from dysfunction and death of pancreatic beta cells leading to insulin insufficiency. Usually developed with a juvenile onset, T1DM is featured by long-term hyperglycemia and progressive macro- and microvascular complications including cardiovascular disease, stroke, nephropathy, neuropathy and retinopathy. Oxidative stress plays a crucial role in the pathogenesis of various diabetic complications (2). There is considerable evidence that supraphysiologic blood sugar contributes to the depletion of oxidant defense system and, therefore, the enhancement of oxidative stress in diabetic patients (10, 26, 27). Additionally, overexpression of proinflammatory cytokines is another pathogenic factor that promotes complications of diabetes. It has been reported that hyperglycemia and oxidative stress trigger activation of nuclear factor κB (NF-κB), which is responsible for the production of several inflammation-regulated genes such as growth factors, proinflammatory cytok-

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Received: February 16, 2011; Revised (Final Version): May 2, 2011; Accepted: May 23, 2011.
 ISSN : 0304-4920. http://www.cps.org.tw

ines and adhesion molecules (3, 8). Further studies have also shown that cytokines promotes apoptotic cascades leading to programmed cell death of islet beta cells, increase oxidative stress and activate the NF-κB signaling pathway (8, 19).

Resveratrol (RSV; 3,5,4'-trihydroxystilbene) is a naturally occurring phytoalexin abundantly produced by grape and berry species. Accumulating evidence has been verified that RSV exerts potent antioxidant competence by scavenging free radicals (6, 20). Further studies show that the cardioprotective and anti-diabetic effects of RSV are mediated by attenuation of oxidative damage (11, 18, 21, 22, 25). RSV also exhibits an anti-inflammatory action through suppression of the NF-κB signaling pathway and cytokine expression (9, 15, 25). In our previous studies, it has been demonstrated that RSV exhibits hypoglycemic and hypolipidemic effects in streptozotocin (STZ)-induced diabetic rats (21). Further investigation has indicated that RSV prevents overproduction of reactive oxygen species (ROS) and impairment of insulin signaling, therefore, correcting diabetes-associated abnormalities (11).

Considering the effects of RSV on amelioration of hyperglycemia, oxidative damage and inflammatory process, we have designed the present study to investigate the effect of RSV against oxidative and inflammatory stress in hepatic and splenic tissuesfficacy of STZ-induced diabetic rats. In evaluation of oxidative stress, superoxide anion content, malondialdehyde (MDA), protein carbonyl levels (PCL) and western blot analysis of expression levels of magnesium superoxide dismutase (Mn-SOD) and nitro-tyrosine (N-Tyr) was performed. The inflammatory severity was determined by utilizing enzyme-linked immunosorbent assays (ELISA) to detect proinflammatory cytokine levels of tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β) and IL-6. Expression of the activated NF-κB subunit, p65, was determined by western blot analysis.

Materials and Methods

Animals and Treatment

This study was performed in accordance to the rules in the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). Male Long-Evans rats (6-7 weeks old, 250-300 g) were used in the experiment, and were maintained in the animal center of Chang Gung University within an environment-controlled room (ambient temperature of 25 ± 1°C and a light-dark period of 12 h) with free access to normal chow and water. The experimental animals were randomly assigned to two groups, the non-diabetic rats (control, CON) and STZ-induced diabetic rats (STZ-DM). In the diabetic group, the male Long-Evans rats were fasted and anesthetized by intraperitoneal injection of pentobarbital at a dosage of 65 mg/kg. Freshly prepared STZ (65 mg/kg) (Sigma-Aldrich, St. Louis, MO, USA) solution was injected intravenously into the femoral vein of the animals. The experimental rats with symptoms such as polyphagia, polydipsia and polyuria together with a blood glucose level above 300 mg/dl were considered diabetic. The blood glucose level was measured using the glucose oxidase method (chemistry analyzer; Auto Analyzer Quik-Lab, Ames, Spain). Two weeks after the onset of diabetes, the diabetic rats were further divided into three subgroups concomitantly treated with the vehicle (STZ-DM), RSV 0.1 mg/kg (DM-R0.1) or RSV 1 mg/kg (DM-R1) for 7 consecutive days. RSV (Sigma-Aldrich) was suspended in saline solution and was administrated by oral gavage. At the end of the RSV treatment course, the rats were euthanized and sacrificed. All the hepatic and splenic tissues and blood samples were preserved at -80°C.

Biological Analysis

In biological analysis, the body weight of each rat was measured before sacrifice. Plasma glucose, insulin, cholesterol and triglyceride levels were determined using commercial kits (R&D, Systems, Minneapolis, MN, USA). All steps were performed according to the manufacturer's instructions. Optical density was read on a plate reader set at 450 nm for glucose, insulin, cholesterol and triglyceride. The contents of glucose, insulin, cholesterol and triglyceride in the samples were calculated from the standard curve multiplied by the dilution factor. The concentrations of glucose, cholesterol and triglyceride were expressed as mg/dl, and the insulin concentration was expressed as μg/l, respectively.

Measurement of Oxidative Stress

Superoxide anion production in hepatic and splenic tissues of diabetic rats was measured by lucigenin-enhanced chemiluminescence with a luminometer (Plate Chameleon, Hidex, Finland) (11, 12). Fresh tissue pieces of liver and spleen were excised and incubated with 0.2 ml of 100 μM lucigenin at 37°C in a 96-well microtiter plate. Lucigenin was counted 10 min later and the superoxide anion content was expressed as relative light unit (RLU)/mg tissue. Lipid peroxidation was determined using the modified thiobarbituric acid reactive substances (TBARS) method (11, 12). 0.2 ml sample homogenate was incubated with in 0.2 ml solution containing 20 mM Tris (pH 7.4), 20.4 mM ferrous sulfate and 0.204 mM

ascorbic acid for 1 h at 37°C. After incubation, 0.4 ml 20% trichloroacetic acid and 0.6 ml 0.67% thiobarbituric acid were pipetted and kept in a water bath for 15 min at 100°C. The mixture was centrifuged at 12,000 × g for 15 min at 4°C. Optical density was read on a plate reader set at 532 nm and MDA concentration was calculated from the standard curve multiplied by the dilution factor and was expressed as nmol/mg protein. Measurement of protein carbonyl group was based on a reaction with 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl solution at room temperature with vortexing every 10 min (12). After addition into 20% trichloroacetic acid and centrifugation at 7,800 × g for 3 min at 4°C, the samples were washed three times in a buffer containing ethanol and ethyl acetate with a ratio of 1:1 (v/v) and the supernatant was discarded after each wash. Sample precipitations were subsequently redissolved in 6 M guanidine solution (pH 2.3) and centrifuged at 7,800 × g for 3 min at 4°C to remove insoluble materials. Optical density was monitored by absorbance at 380 nm and PCL was expressed as pmol/mg protein.

Western Blot Analysis

Sample lysis was performed for 30 min at 4°C with a lysis buffer containing 50 mM Tris-HCl (pH 7.4), 50 mM β-glycerophosphate, 20 mM sodium fluoride, 2 mM sodium orthovanadate, 2 mM EDTA, 1 mM phenylmethanesulfonyl fluoride, 1% 2-mercaptoethanol, and lysates were centrifuged at 12,000 × g for 20 min at 4°C to remove nuclei and undisrupted cells. Protein concentration was determined using the Bio-Rad protein assay solution with BSA as the standard. Sample proteins were loaded and run on a 10% SDS-polyacrylamide gel in Tris-glycine electrophoresis buffer [25 mM Tris (pH 8.3), 240 mM glycine, and 0.4% SDS]. Proteins were transferred to a nitrocellulose membrane in a buffer containing 192 mM glycine, 25 mM Tris (pH 8.3), and 20% methanol at 200 mA for 2 h in a water-cooled transfer apparatus. The membrane was blocked in a blocking buffer, TBST [20 mM Tris (pH 7.4), 150 mM sodium chloride, and 0.5% Tween-20] containing 5% nonfat milk, at room temperature for 1 h. The membrane was then probed overnight at 4°C with polyclonal antibodies against Mn-SOD, N-Tyr (Upstate Biotechnology, Lake Placid, NY, USA) and NF-κB (Chemicon, Temecula, CA, USA) in TBST. After the membrane was washed four times with 10 min intervals in TBST, the membrane was subsequently incubated with a goat anti-mouse IgG (Upstate Biotechnology) for N-Tyr and goat anti-rabbit IgG for Mn-SOD and NF-κB p65 subunit, conjugated with horseradish peroxidase diluted to 1:10000 in TBST for 1 h at room temperature. After the membrane was washed four times with 10 min intervals in TBST, the bound antibodies were detected using a chemiluminescent substrate kit (Amersham, Pittsburgh, PA, USA).

ELISA for Proinflammatory Cytokines

To determine the concentrations of proinflammatory cytokines TNF-α, IL-1β and IL-6 in hepatic and splenic tissues, double antibody sandwich ELISA (R&D, Systems) was applied according to the manufacture's instructions. Optical density was read on a plate reader set at 450 nm for TNF-α, IL-1β and IL-6. The concentrations of TNF-α, IL-1β and IL-6 in the samples were calculated from the standard curve multiplied by the dilution factor and were expressed as pg/ml.

Statistical Analysis

The values obtained in the experiment were expressed as means ± standard error of the mean (SEM). Following the performance by one-way analysis of variance (ANOVA), the data were analyzed by using Student's *t*-test for the determination of the significance of the difference between groups. A value of $P < 0.05$ was considered to be statistically significant.

Results

Animal Characteristics

The metabolic parameters of the animal groups are summarized in Table 1. A remarkable loss in body weight was found in the STZ-DM group compared with that of the CON group (431.36 ± 13.28 *vs*. 344.00 ± 14.24 g in the CON group, $P < 0.05$); no significant change of body weight was observed in the RSV-treated groups. The rats in the STZ-DM group had a significantly higher plasma glucose level (530.15 ± 61.47 *vs*. 138.67 ± 9.94 mg/dl in the CON group, $P < 0.05$) and lower plasma insulin level (0.32 ± 0.06 *vs*. 1.74 ± 0.30 μg/l in the CON group, $P < 0.05$) than the rats in the CON group. No significant change was revealed in the plasma triglyceride and cholesterol levels. Administration of RSV significantly reduced the plasma glucose level (530.15 ± 61.47 *vs*. 325.51 ± 31.95 mg/dl in the DM-R1 group, $P < 0.05$) and elevated the plasma insulin level (0.32 ± 0.06 *vs*. 0.90 ± 0.14 μg/l in the DM-R1 group, $P < 0.05$). Additionally, RSV significantly reduced plasma cholesterol concentration in the diabetic group (74.24 ± 3.86 *vs*. 53.12 ± 6.77 mg/dl in the DM-R0.1 group, $P < 0.05$; 74.24 ± 3.86 *vs*. 51.39 ± 6.92 mg/dl in the DM-R1 group, $P < 0.05$). The concentration of plasma triglyceride was not altered in the RSV-treated groups. Therefore, RSV exhibited hypoglycemic, hyperin-

Table 1. The biochemical parameters of CON, STZ-DM, DM-R0.1 and DM-R1 rats

	CON (n = 11)	STZ-DM (n = 8)	DM-R0.1 (n = 9)	DM-R1 (n = 11)
Body weight (g)	431.36 ± 13.28	344.00 ± 14.24*	324.00 ± 16.03	352.73 ± 10.63
Plasma glucose (mg/dl)	138.67 ± 9.94	530.15 ± 61.47*	397.71 ± 52.26	325.51 ± 31.95†
Plasma insulin (μg/l)	1.74 ± 0.30	0.32 ± 0.06*	0.52 ± 0.11	0.90 ± 0.14†
Plasma cholesterol (mg/dl)	70.02 ± 8.00	74.24 ± 3.86	53.12 ± 6.77†	51.39 ± 6.92†
Plasma triglycerides (mg/dl)	46.23 ± 9.78	48.49 ± 6.37	46.32 ± 6.82	48.09 ± 7.64

Values are expressed as means ± SEM (n = 8~20 per group). CON, non-diabetic control; STZ-DM, streptozotocin-induced diabetes; DM-R0.1, DM treated with RSV (0.1 mg/kg/day) for 7 days; DM-R1, DM treated with RSV (1 mg/kg/day) for 7 days. *, $P < 0.05$ *vs*. control; †, $P < 0.05$ *vs*. STZ-DM.

sulinemic and hypocholesteremic effects in STZ-induced type 1 diabetic rats.

The Effect of RSV on Oxidative Stress in Diabetic Liver

The effect of RSV on oxidative stress in the hepatic tissues of diabetic rats is presented in Fig. 1. When compared with the CON group, a significant increase in the superoxide anion content (8.89 ± 2.16 *vs*. 4.57 ± 0.94 RLU/10 min/mg tissue in the CON group, $P < 0.05$) was shown in the hepatic tissue of STZ-DM rats. The MDA level in the hepatic tissues of the STZ-DM rats showed no remarkable difference when compared with the CON group, while PCL was significantly increased (218.63 ± 30.61 *vs*. 82.73 ± 16.30 pmol/mg protein in the CON group, $P < 0.05$) in the liver of the diabetic group. The level of N-Tyr in the STZ-DM group was significantly increased (111.90 ± 4.11% of the CON group, $P < 0.05$). Additionally, the hepatic protein expression of Mn-SOD in the STZ-DM group was remarkably increased (119.84 ± 7.95% of the CON group, $P < 0.05$) in comparison with the CON group. RSV administration markedly decreased superoxide anion (8.89 ± 2.16 *vs*. 4.30 ± 0.50 RLU/10 min/mg tissue in the DM-R0.1 group, $P < 0.05$; 8.89 ± 2.16 *vs*. 4.75 ± 0.75 RLU/10 min/mg tissue in the DM-R1 group, $P < 0.05$), PCL (218.63 ± 30.61 *vs*. 108.29 ± 17.61 pmol/mg protein in the DM-R0.1 group, $P < 0.05$), and Mn-SOD expression (118.65 ± 7.36% *vs*. 82.19 ± 12.10% in the DM-R1 group, $P < 0.05$) in the liver of the diabetic rats. No notable change of the MDA level and N-Tyr expression was shown in the liver of RSV-treated groups when compared with the diabetic group. Therefore, RSV administration significantly attenuated oxidative stress by reducing superoxide anion, PCL and Mn-SOD protein expression in the hepatic tissues of diabetic rats.

The Effect of RSV on Inflammatory Response in Diabetic Liver

The effect of RSV on inflammatory activity in the liver of diabetic rats is shown in Fig. 2. The STZ-DM rats presented a significant increase of activated NF-κB subunit p65 (136.26 ± 14.36% of the CON group, $P < 0.05$) and IL-1β levels (284.15 ± 44.87 *vs*. 158.86 ± 7.06 pg/mg protein in the CON group, $P < 0.05$) in the hepatic tissues when compared with control rats. No marked difference of TNF-α and IL-6 concentrations was found between the diabetic and non-diabetic groups. RSV administration markedly reduced the activated form of NF-κB (136.26 ± 14.36% *vs*. 78.39 ± 11.94% in the DM-R1 group, $P < 0.05$) in the liver of the diabetic rats. RSV also decreased hepatic cytokine IL-1β level (284.15 ± 44.87 *vs*. 174.36 ± 16.54 pg/mg protein in the DM-R0.1 group, $P < 0.05$). No significant alteration was found in hepatic cytokine TNF-α and IL-6 levels after RSV supplementation in the diabetic rats. Our data showed that administration of RSV significantly inhibited inflammatory activities by reducing NF-κB p65 expression and reduced the IL-1β cytokine level in the hepatic tissues of diabetic rats.

Effect of RSV on Oxidative Stress in Diabetic Spleen

The effect of RSV on oxidative stress in the splenic tissues of diabetic rats is shown in Fig. 3. In comparison with the CON group, a significant enhancement of the superoxide anion content was found in the splenic tissues of diabetic rats (19.05 ± 6.00 *vs*. 7.85 ± 1.86 RLU/10 min/mg tissue in the CON group, $P < 0.05$). The MDA level (1179.36 ± 136.39 *vs*. 831.19 ± 27.03 nmol/mg protein in the CON group, $P < 0.05$) and PCL (183.15 ± 19.11 *vs*. 119.98 ± 20.40 pmol/mg protein in the CON group, $P < 0.05$) were significantly increased in the diabetic spleen when compared with the CON group. The N-Tyr expression in the STZ-DM group did not show a significant alteration when compared with the control rats. Additionally, the protein expression of Mn-SOD in the diabetic group was significantly reduced (73.11 ± 11.13% of the CON group, $P < 0.05$) when compared with the CON group. RSV treatment remarkably reduced superoxide anion (19.05 ± 6.00 *vs*. 5.23 ± 1.03 RLU/10 min/mg tissue in the DM-R0.1 group, $P < 0.05$; 19.05 ± 6.00 *vs*. 6.82 ±

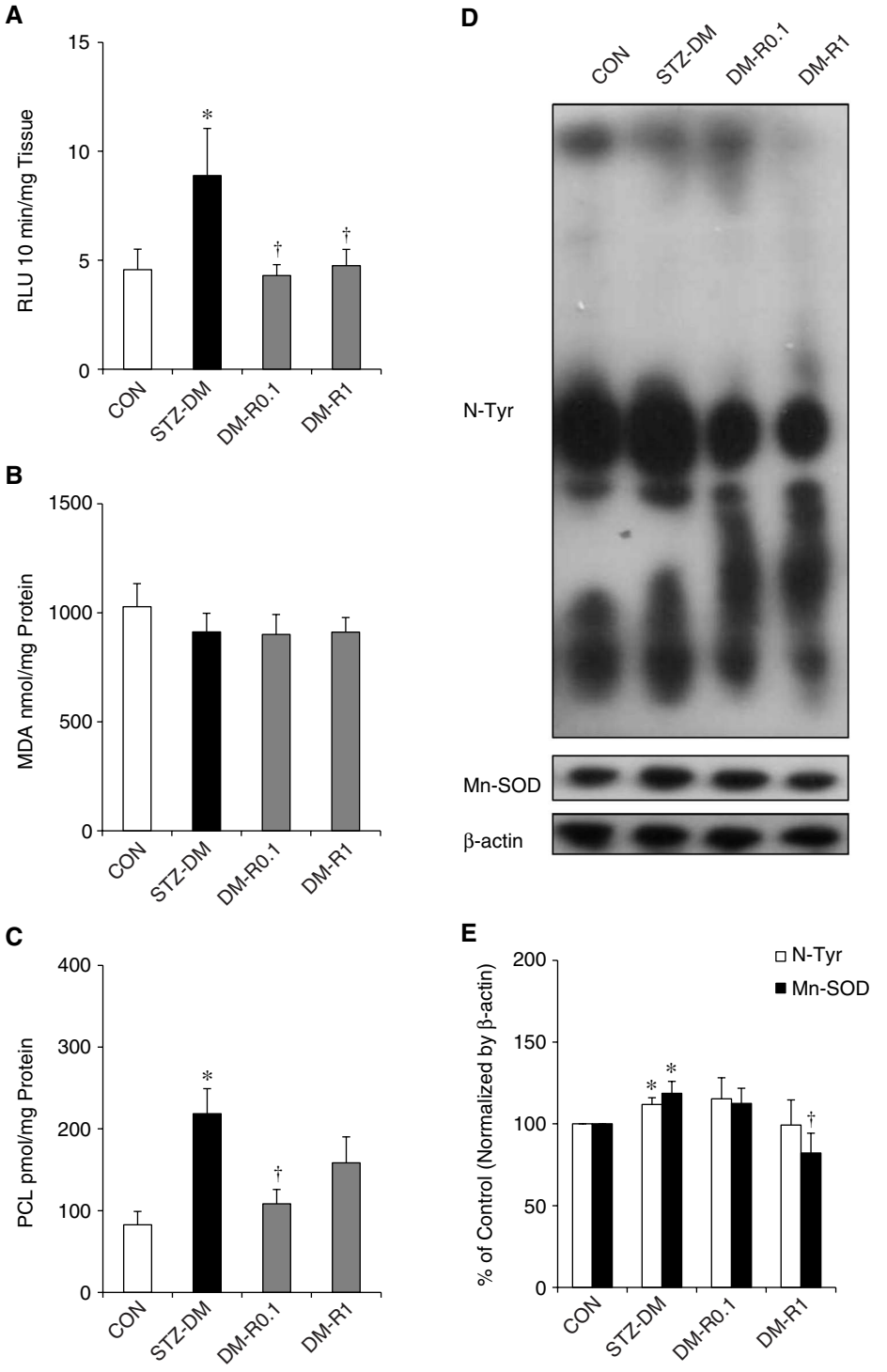

Fig. 1. The effect of RSV on superoxide anion production, lipid peroxidation, protein carbonyl level, and protein expression levels of N-Tyr and Mn-SOD in the hepatic tissues of STZ-induced diabetic rats. Production of superoxide anion was determined using lucigenin-enhanced chemiluminescence. MDA level was measured using the TBARS method for evaluation of lipid peroxidation. Concentration of carbonyl protein was measure by the DNPH method. Equal amounts of proteins were resolved on 10% SDS-PAGE and blotted with N-Tyr and Mn-SOD antibodies in western blot analysis. **A**: Superoxide anion production among the study groups. **B**: MDA concentrations among the groups. **C**: The contents of carbonyl protein. **D**: Western blot analysis of N-Tyr and Mn-SOD. **E**: Densitometeric values normalized by β-actin of N-Tyr and Mn-SOD protein levels among the study groups. Results are expressed as means ± SEM (n = 5 per group). *, $P < 0.05$ *vs*. control. †, $P < 0.05$ *vs*. STZ-DM. RLU, relative light unit; MDA, malondialdehyde; PCL, protein carbonyl level; N-Tyr, nitro-tyrosine; Mn-SOD, manganese superoxide dismutase; CON, non-diabetic control; STZ-DM, streptozotocin-induced diabetes; DM-R0.1, DM treated with RSV (0.1 mg/kg/day) for 7 days; DM-R1, DM treated with RSV (1 mg/kg/day) for 7 days.

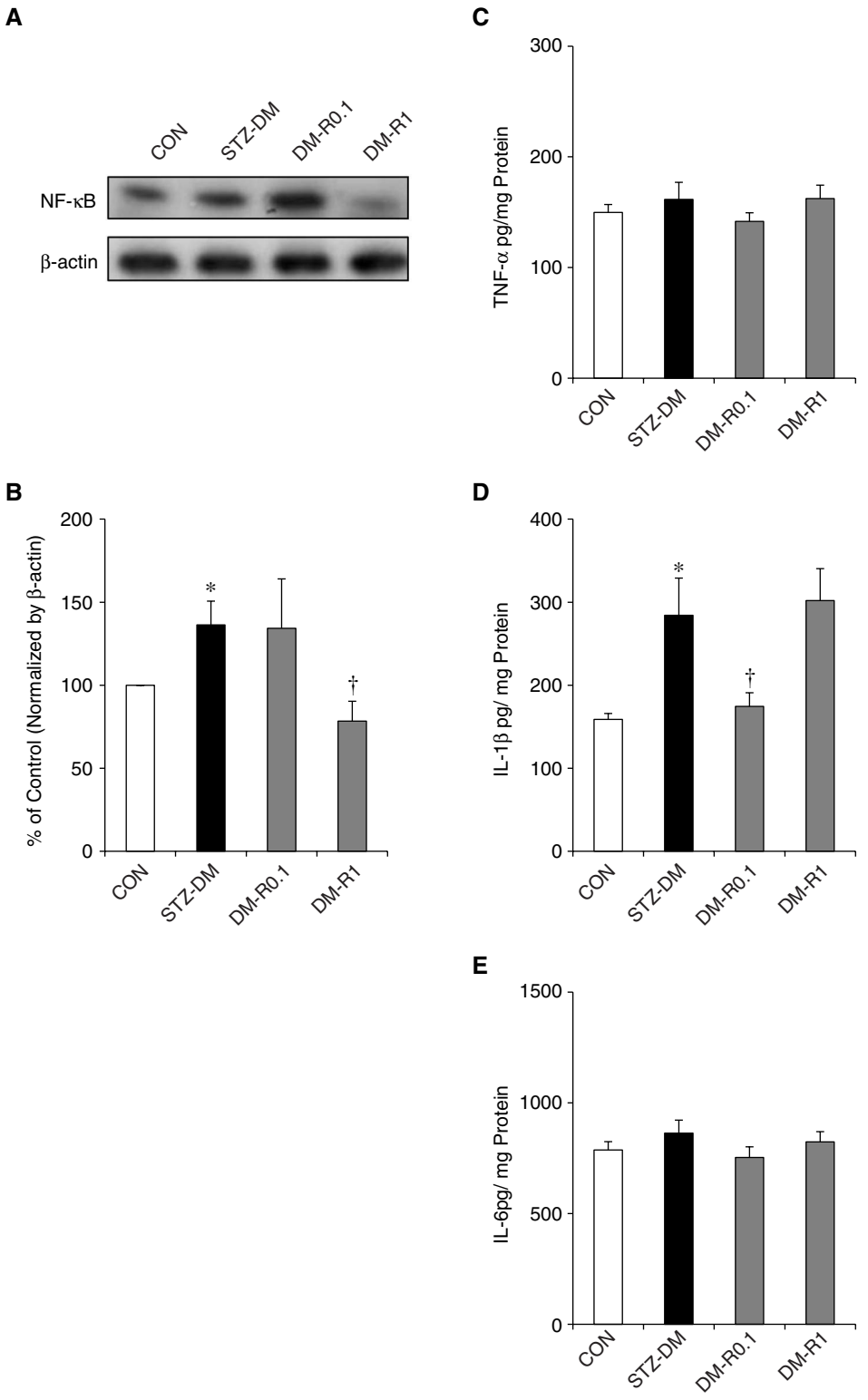

Fig. 2. The effect of RSV on protein expression of NF-κB and concentrations of TNF-α, IL-1β and IL-6 in the hepatic tissues of STZ-induced diabetic rats. Equal amounts of proteins were resolved on 10% SDS-PAGE and blotted with an anti-NF-κB p65 antibody in western blot analysis. Samples were processed to measure IL-1β, TNF-α or IL-6 using competitive ELISA. **A**: Western blot analysis of NF-κB. **B**: Densitometeric values normalized by β-actin of the NF-κB protein level. **C**: TNF-α cytokine levels. **D**: IL-1β cytokine levels. **E**: IL-6 cytokine levels. All data were presented as means ± SEM (n = 5 per group). *, $P < 0.05$ *vs*. control. †, $P < 0.05$ *vs*. STZ-DM. NF-κB, nuclear factor κB; TNF-α, tumor necrosis factor α; IL-1β, interleukin 1β; IL-6, interleukin 6; CON, non-diabetic control; STZ-DM, streptozotocin-induced diabetes; DM-R0.1, DM treated with RSV (0.1 mg/kg/day) for 7 days; DM-R1, DM treated with RSV (1 mg/kg/day) for 7 days.

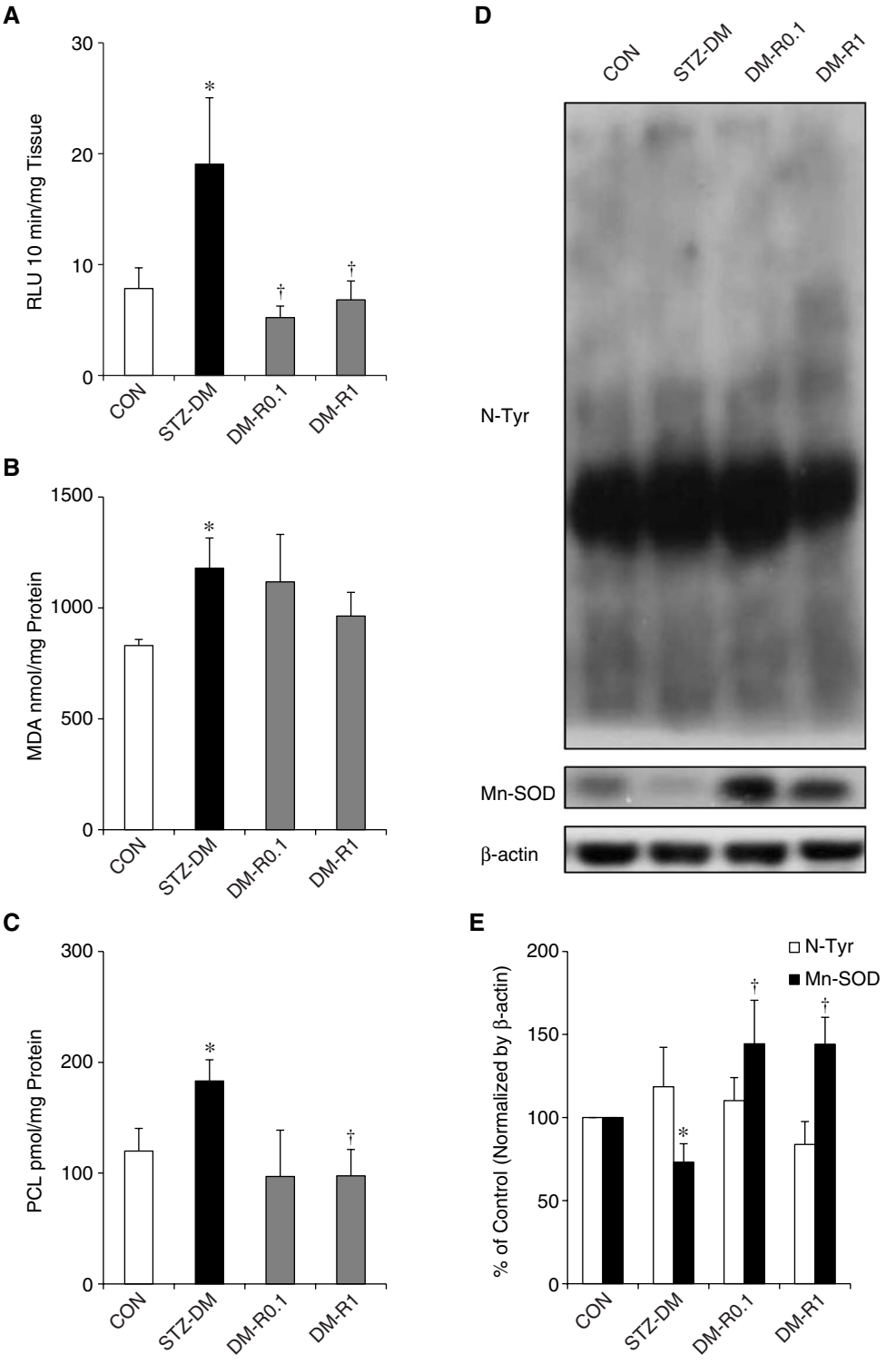

Fig. 3. The effect of RSV on superoxide anion production, lipid peroxidation, protein carbonyl level and protein expression levels of N-Tyr and Mn-SOD in the splenic tissues of STZ-induced diabetic rats. Production of superoxide anion was determined using lucigenin-enhanced chemiluminescence. MDA level was measured using the TBARS method for evaluation of lipid peroxidation. Concentration of carbonyl protein was measure by the DNPH method. Equal amounts of proteins were resolved on 10% SDS-PAGE and blotted with anti-N-Tyr and anti-Mn-SOD antibodies, respectively, in western blot analysis. **A**: The superoxide anion production. **B**: MDA concentrations. **C**: Contents of carbonyl protein. **D**: Western blot analysis of N-Tyr and Mn-SOD. **E**: Densitometeric values normalized by β-actin of N-Tyr and Mn-SOD proteins levels. Results are expressed as means ± SEM (n = 5 per group). *, $P < 0.05$ *vs*. control. †, $P < 0.05$ *vs*. STZ-DM. See legend to Fig. 1 for explanation of abbreviations used.

1.70 RLU/10 min/mg tissue in the DM-R1 group, $P < 0.05$) and PCL (183.15 ± 19.11 *vs*. 97.49 ± 23.87 pmol/mg protein in the DM-R1 group, $P < 0.05$) and increased Mn-SOD expression (73.11 ± 11.13% *vs*. 144.38 ± 26.22% in the DM-R0.1 group, $P < 0.05$; 73.11 ± 11.13% *vs*. 144.14 ± 16.21% in the DM-R1 group, $P < 0.05$). The MDA level and N-Tyr expression were not significantly altered in the splenic tissues of RSV-treated groups. Therefore, RSV supplementation significantly ameliorated oxidative stress by reducing superoxide anion and PCL, and increasing Mn-SOD protein expression in the splenic tissues of diabetic rats.

The Effect of RSV on Inflammatory Response in Diabetic Spleen

The effect of RSV on splenic inflammation is presented in Fig. 4. There were no notable changes of activated unit of NF-κB p65 expression, and in the TNF-α, IL-1β and IL-6 cytokine concentrations in the STZ-DM group when compared with the CON group. The NF-κB p65 expression was remarkably reduced in the splenic tissues of the RSV-treated group (134.26 ± 18.17% *vs*. 57.72 ± 14.14% in the DM-R1 group, $P < 0.05$). Additionally, the levels of TNF-α (298.13 ± 19.19 *vs*. 483.45 ± 67.08 pg/mg protein in the DM-R1 group, $P < 0.05$) and IL-6 (716.04 ± 61.15 *vs*. 1111.34 ± 124.45 pg/mg protein in the DM-R1 group, $P < 0.05$) were significantly increased after RSV administration in the diabetic spleen. There was also an increasing tendency in the IL-1β cytokine level in the splenic tissues of RSV-treated diabetic rats. In summary, RSV treatment decreased NF-κB p65 expression, but elevated TNF-α and IL-6 cytokine levels in the splenic tissues of diabetic rats.

Discussion

The current study indicated that: [1] in STZ-induced type 1 diabetic rats with hyperglycemia and hypoinsulinemia, RSV administration remarkably attenuated high blood sugar and increased plasma insulin levels; [2] in the diabetic animal model, oxidative stress was significantly reduced after RSV supplementation; [3] although numerous studies have indicated that RSV possessed an anti-inflammatory potential through the NF-κB signaling pathway, our data revealed that RSV promoted inflammatory response and cytokine overproduction in a NF-κB-independent mechanism in the splenic tissues of STZ-induced diabetic rats.

In physiological condition, excessive generation of ROS could be rapidly detoxified by antioxidant enzymes. In T1DM, however, hyperglycemia provoked overproduction of ROS and impairment of antioxidant enzymes leading to oxidative stress and organ dysfunction (8, 14). Our results further indicated the effect of hyperglycemia-related oxidative stress was tissue-specific, in which hepatic Mn-SOD was overexpressed but splenic Mn-SOD expression was reduced.

It has been known that oxidative stress stimulates the stress-activated signaling pathway in which NF-κB plays a major role. Hyperglycemia and oxidative stress trigger NF-κB activation, producing cytokines and aggravating inflammatory activities. NF-κB also modulates expression of adhesion molecules and growth factor-related genes, which in turn activates NF-κB signal itself (8). As oxidative stress and inflammatory process develop and persist with poor glycemic control, advanced complications of T1DM ultimately occur (16).

It is wide accepted that RSV performs as a free radical scavenger due to its chemical structure. Further, RSV is recognized as a potent antioxidant based on its subsequent activation of antioxidant enzymes (6). There is considerable evidence providing the competence of RSV against oxidative stress in a variety of hyperglycemia-exposed organs, including heart (11, 22), liver (14, 17), kidney (20), skeletal muscle (7), vascular endothelium (24, 25) and nervous tissues (1). In our experimental results, RSV remarkably ameliorated oxidative stress through reducing superoxide anion content, preventing protein oxidation and reversing Mn-SOD expression in the hepatic and splenic tissues of STZ-induced diabetic rats.

The suppressive effects of RSV on inflammatory response have been investigated (5). The anti-inflammatory properties of RSV are attributed to inhibition of NF-κB, therefore, blocking the release of proinflammatory cytokines and amplification of inflammation *via* inhibition of downstream inflammatory mediators. Recent studies have, nevertheless, revealed the pro-inflammatory potential of RSV *via* enhancement of NF-κB activity (4, 23). Our results indicated that RSV reduced IL-1β cytokine and NF-κB expression levels in the hepatic tissues, but increased TNF-α and IL-6 cytokine levels with NF-κB down-regulation in the splenic tissues of STZ-induced diabetic rats. The findings deviated from our original expectation that RSV should suppress inflammatory activity in hyperglycemia-exposed tissues. One explanation to the discrepancy is that RSV may activate other subunits rather than p65 in the NF-κB complex to trigger inflammatory cascades. It is also possible that RSV modulated mitogen-activated protein kinase (MAPK) signaling pathways and estrogen receptor-mediated cascades to amplify inflammation in an NF-κB-independent manner (13). The mechanism of RSV on inflammation requires further research.

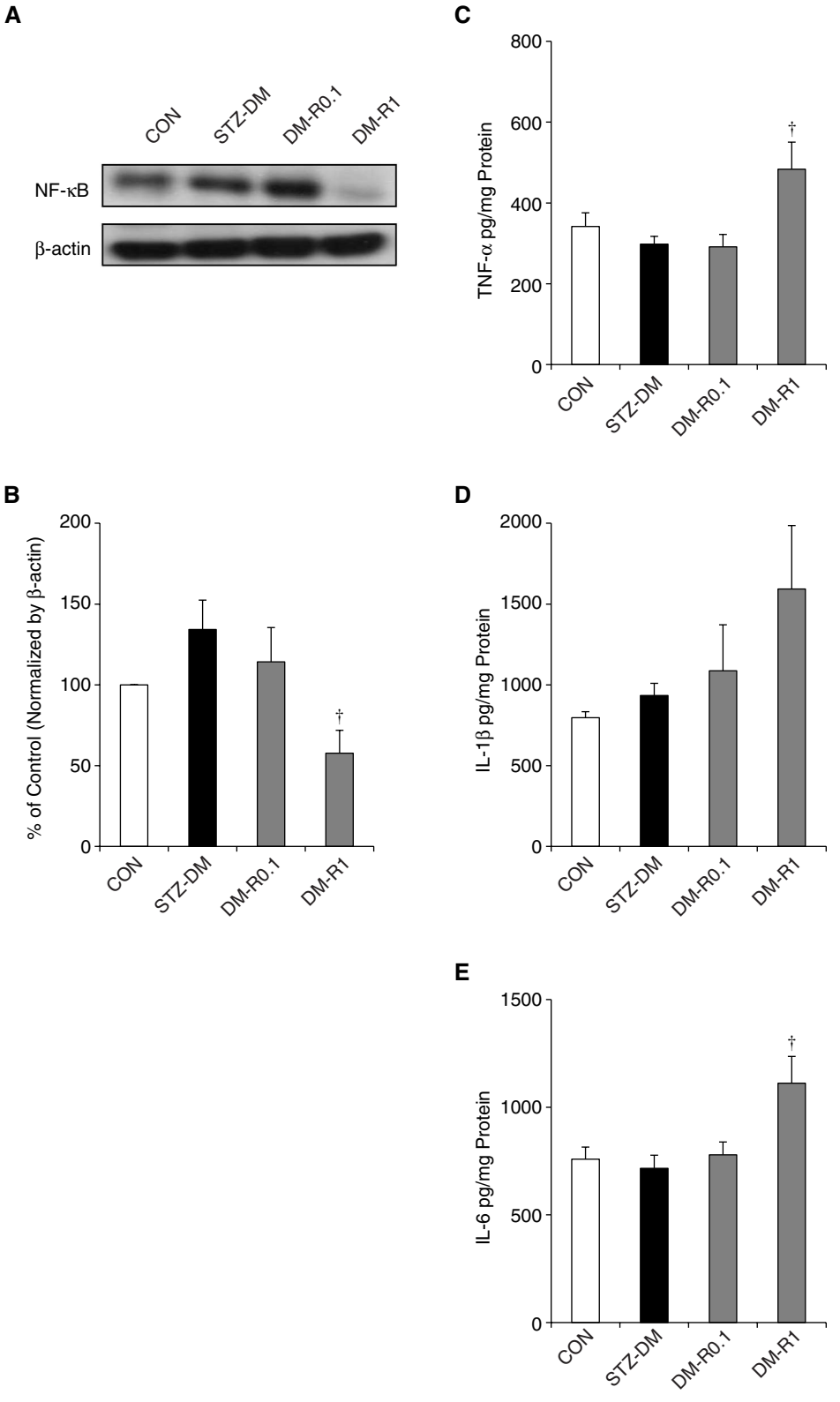

Fig. 4. The effect of RSV on protein expression of NF-κB and concentrations of TNF-α, IL-1β and IL-6 in the splenic tissues of STZ-induced diabetic rats. Equal amounts of proteins were resolved on 10% SDS-PAGE and blotted with an anti-NF-κB p65 antibody in western blot analysis. Samples were processed to measure IL-1β, TNF-α or IL-6 using competitive ELISA. **A**: Western blot analysis of NF-κB. **B**: Densitometeric values normalized by β-actin of NF-κB protein levels. C: TNF-α cytokine level. **D**: IL-1β cytokine levels. **E**: IL-6 cytokine levels. All data are presented as means ± SEM (n = 5 per group). *, $P < 0.05$ *vs*. control. †, $P < 0.05$ *vs*. STZ-DM. See legend to Fig. 2 for explanation of abbreviations used.

In summary, our results indicated ameliorative effects of RSV on oxidative stress in the liver and spleen of STZ-induced diabetic rats. Further, RSV reduced hepatic cytokine expression in an NF-κB-dependent mechanism, but increased splenic cytokine levels without NF-κB signal intervention in T1DM. If and how RSV influences inflammatory signals remain controversial. Our findings suggest that RSV may act as a potent antioxidant, but possesses a proinflammatory potential in specific situations in T1DM.

Acknowledgments

This work was financially supported by research grants from Chang Gung Memorial Hospital (CMRPD 180191) and the National Science Council (NSC 97-2320-B-182-022-MY3) of Taiwan to Dr. Li-Man Hung.

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