Biochemical and Biophysical Research Communications 399 (2010) 98–103

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Dietary restriction suppresses inflammation and delays the onset of stroke in stroke-prone spontaneously hypertensive rats

Tsuyoshi Chiba, Osamu Ezaki *

Nutritional Science Program, National Institute of Health and Nutrition, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan

ARTICLE INFO

Article history:
Received 6 July 2010
Available online 17 July 2010

Keywords:
Dietary restriction
Inflammation
Macrophage
Stroke
SHRSP

ABSTRACT

Subjects with high blood levels of inflammatory markers and patients with chronic inflammatory disorders are at high risk for stroke. Dietary restriction (DR) suppresses systemic inflammation to deter age-related chronic diseases. To examine whether DR delays the onset of stroke, 10-week-old stroke-prone spontaneously hypertensive rats (SHRSP) were assigned to either a control (*ad libitum*) or DR (50% diet of control) group, and day of stroke onset and lifespan were observed. DR markedly delayed the onset of stroke in SHRSP compared to control without affecting blood pressure. Day of stroke onset (median) in the control group was 34 days, whereas it was 70 days in the DR group. After 2 weeks of DR and before the onset of stroke, plasma levels of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) and their mRNA expression levels in adipose tissue were significantly lower in the DR rats than in the control rats. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) mRNA expression levels in cerebrovascular endothelial cells (CVECs), and macrophage infiltration into brain were lower in the DR rats than in the control rats. IL-1β and TNF-α treatment in CVECs increased MCP-1, C-reactive protein, ICAM-1, and VCAM-1 mRNA and their protein levels *in vitro*. In conclusion, suppression of inflammation in response to DR may lead to a delay in the onset of stroke independent of any effect on blood pressure in SHRSP.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Inflammatory processes are recognized to play a central role in the pathogenesis of atherosclerosis and its complications. In clinical settings, plasma levels of inflammatory bio-markers (cytokines, C-reactive protein [CRP], chemokines) have been found to be associated with future cardiovascular risk [1]. Administration of anti-inflammatory drugs may prevent stroke in humans and in animal models. In healthy persons without hyperlipidemia but with elevated high-sensitivity CRP level, rosuvastatin, which lowers high-sensitivity CRP as well as cholesterol, reduced the incidence of stroke and myocardial infarction by 50% relative to placebo [2].

Stroke-prone spontaneously hypertensive rats (SHRSP), a unique genetic model of stroke [3], have previously been used to examine the contributions of inflammation to stroke. In SHRSP fed a high-salt diet, rosuvastatin treatment significantly delayed the onset of stroke and attenuated the transcription of inflammatory bio-markers (monocyte chemoattractant protein-1 [MCP-1], transforming growth factor-β1, interleukin-1β [IL-1β], and tumor necrosis factor-α [TNF-α]) [4]. Pioglitazone, an agonist of peroxisome proliferator-activated receptor-γ, reduced the risk of recurrent stroke in patients with type 2 diabetes [5]. In SHRSP, pioglitazone delayed the onset of stroke by improving vascular endothelial dysfunction, inhibiting brain inflammation, and reducing oxidative stress [6]. A low dose of acetylsalicylic acid (aspirin) delayed the onset of stroke in SHRSP with suppression of inflammation [7].

Dietary restriction (DR) has been shown to suppress systemic inflammation and thus deter age-related chronic diseases such as atherosclerosis, cancer, diabetes, obesity, and metabolic syndrome [8]. Pro-inflammatory mediators such as IL-1β, IL-6, TNF-α, and CRP are attenuated by DR [9,10]. Diet-restricted human subjects have shown significantly lower levels of TNF-α and CRP than have healthy, aged-matched controls [11]. During war or severe economic crisis where marked shortages of food persisted for several years, incidence of stroke was reduced [12,13]. Therefore, DR may prevent stroke in humans and rats by lowering systemic inflammation. However, SHRSP fed a low-protein diet (5% calories from protein) *ad libitum* showed acceleration in the onset of stroke [14], making it unclear whether DR, which reduces systemic inflamma-

Abbreviations: CVECs, cerebrovascular endothelial cells; DR, dietary restriction; ICAM, intercellular adhesion molecule; IL, interleukin; MCP, monocyte chemoattractant protein; NF-κB, nuclear factor-kappa B; ROS, reactive oxygen species; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone spontaneously hypertensive rats; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule.

* Corresponding author. Fax: +81 3 3207 3520.
E-mail addresses: tyschiba@nih.go.jp (T. Chiba), ezaki@nih.go.jp (O. Ezaki).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.07.048

tion, delays or accelerates stroke. We therefore conducted studies of DR in SHRSP to answer this question.

2. Materials and methods

2.1. Experimental protocols

Male SHRSP/Izm were purchased from Japan SLC Inc. (Shizuoka, Japan) and maintained under specific pathogen-free conditions in a temperature-controlled room (22 ± 2 °C) with a 12-h light/dark cycle. Rats had free access to a normal laboratory diet (CE-2: CLEA Japan Inc., Tokyo, Japan) and water until 10 weeks of age, when experiments began. Control rats were fed an experimental diet under *ad libitum* conditions; DR rats were given 50% of the diet consumed by the control group at 10:00 am each day. Experimental diet was purchased from Research Diet Inc. (New Brunswick, NJ), and composition of the control diet is shown in our previous report [14]. All rats were given drinking water supplemented with 1% NaCl at the beginning of DR. Each group comprised eight rats, each of which was kept in a separate steel cage and monitored for neurological symptoms and physiological changes to detect the onset of stroke.

To determine physiological characteristics, rats were killed at 10:00 am after 2 weeks of DR and before the onset of stroke. Control rats were fasted overnight to take into account the acute effects of fasting in DR rats (the authors wanted to compare the chronic effects of DR with *ad libitum* feeding). Blood was drawn from the abdominal aorta, and plasma was prepared rapidly. Tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C until RNA isolation. All animal experiments were conducted with the approval of the National Institute of Health and Nutrition Ethics Committee on Animal Research.

2.2. Food intake, body weight, and blood pressure

Food intake, body weight, and blood pressure were measured as described in our previous study [14].

2.3. Plasma chemistry

Plasma IL-1β, IL-6, and TNF-α levels were determined with Quantikine rat IL-1β, IL-6, or TNF-α immunoassay kits, respectively (R&D Systems, Inc., Minneapolis, MN). Plasma MCP-1 level was determined with Endogen Rat MCP-1 ELISA Kit (Pierce Biotechnology, Inc., Rockford, IL).

2.4. Onset of stroke

Onset of stroke was assessed by the appearance of neurologic symptoms (hyperkinetic/hypokinetic behaviors, hyper/hyposensitivity [manipulation], or proleg lift, piloerection) and physiological changes (decrease in body weight or food intake or increase in drinking water intake) as described previously [14]. Two people checked for these symptoms at 10:00 am every day.

2.5. Cerebrovascular endothelial cells

Cerebrovascular endothelial cells (CVECs) were harvested in the manner of previous reports with some modifications [15–17]. Four-week-old male SHRSP (for cell culture) or SHRSP with 2 weeks of DR (for gene expression) were killed and brains were removed. The whole brain of each rat (about 2 g) was homogenized with a Teflon-pestle homogenizer in 10 ml of 0.32 M sucrose buffer (0.32 M sucrose, 3 mM HEPES, 10 μM $CaCl_2$, pH 7.4). Homogenate was centrifuged (1000*g*, 10 min), and the pellets were re-suspended with 10 ml of 0.32 M sucrose buffer. The suspension was centrifuged (100 *g*, 15 s), and the pellets were re-suspended with 0.4 ml of 0.32 M sucrose buffer. This suspension was centrifuged (100 *g*, 15 s), and the collected supernatant was re-centrifuged (200 *g*, 1 min). The pellet (microvascular-rich fraction) was incubated with DMEM containing dispase (1000 protease units/ml) for 30 min. After incubation, cells were centrifuged (1000*g*, 10 min), and the pellet was re-suspended in 1 ml of 0.32 M sucrose buffer. CVECs were isolated with Ficoll gradient (7.5%, 12%, 18%, and 32%) ultracentrifugation (53,000 *g*, 30 min) and collected as cells between 18% and 32% Ficoll. The acetylated low-density lipoprotein labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindo-carbocyanine perchlorate (DiI-Ac-LDL; Biomedical Technologies, Inc., Stoughton, MA) uptake, a maker of endothelial cells, was confirmed in prepared CVECs. CVECs were washed with PBS and re-suspended with Endothelial Cell Basal Medium-2 (Lonza, Walkersville, MD) for cell culture or TRIzol Reagent for total RNA extraction. For *in vitro* study, cells were plated onto a gelatin-coated 90-mm dish and used within 10 passage numbers.

To examine the effects of inflammatory cytokine on expression of adhesion molecules, CVECs prepared from 4-week-old SHRSP were plated into a collagen-coated 60-mm dish and cultured until confluent with Endothelial Cell Basal Medium-2 (5% FCS). The CVECs were then growth-arrested with serum-free media for 24 h and incubated with IL-1β, IL-6, or TNF-α (10 ng/ml) in serum-free DMEM for 72 h. After the incubation periods, total RNA and protein were extracted, and mRNA expression levels were measured by quantitative RT-PCR and protein levels by Western blot analysis.

2.6. Quantitative RT-PCR

Total RNA was extracted with TRIzol Reagent (Invitrogen Co., Carlsbad, CA) and reverse transcribed with Omniscript RT Kit (QIAGEN, GmbH, Germany) and oligo (dT) primers. Reactions were performed with SYBR Green PCR Master Mix and a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). The results are expressed as the copy number ratio of the target mRNA to GAPDH (for CVECs) or β-actin (for tissues and cultured CVECs) mRNA. The rat-specific primer pairs used are listed in Supplementary Table 1.

2.7. Western blot analysis

Whole protein from CVECs was extracted with PRO-PREP (iNtRON Biotechnology Inc., Gyeonggi-do, Korea) according to the manufacturer's instructions. CVECs (90-mm dish) were lysed in 600 μl PRO-PREP and incubated for 30 min at −20 °C. Samples were then centrifuged (15,000 *g*, 5 min, 4 °C), and supernatant was collected as the whole protein fraction. Protein in the conditioned media was concentrated using an Amicon Ultra-4 centrifugal filter (Millipore Co., Billerica, MA). Protein concentration was measured with Dc Protein Assay Reagent (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. Protein (10 μg) separated by SDS–PAGE (12.5% gel) was transferred onto Immun-Blot PVDF Membrane (Bio-Rad Laboratories) and immunoblotted with specific primary antibodies: MCP-1 (sc-28879), CRP (sc-73864), ICAM-1 (sc-74097), VCAM-1 (sc-8304), and β-actin (sc-47778) (1:200–500 dilution, Santa Cruz Biotechnology Inc., Santa Cruz, CA). Peroxidase-conjugated anti-rabbit IgG or anti-mouse IgG (GE Healthcare, Buckinghamshire, UK) (1:2000 dilution) was used as the secondary antibody. Bands were visualized by enhanced chemiluminescence system (GE Healthcare), and density of each band was analyzed with Image J 1.41 software (National Institute of Health). An equal amount of loaded protein was verified using anti-β-actin antibody.

2.8. Statistical analysis

Data are presented as the mean ± SEM. Comparisons of data between two groups were made by unpaired Student's *t*-test (Statview 5.0, Abacus Concepts, Piscataway, NJ). Comparisons of data from multiple groups were made by one-way ANOVA with a Bonferroni post hoc test. A Kaplan–Meier curve was obtained for the onset of stroke and survival curves, and the comparison of groups was performed using the log-rank test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. DR delays the onset of stroke and extends lifespan in SHRSP

SHRSP were assigned to either the control (*ad libitum*) or DR group. The baseline food intakes of DR rats were reduced by 50%, and the onset of stroke and lifespan was monitored. DR rats first slightly decreased and then gradually increased in weight throughout the experimental period (Fig. 1A). Body weight in control rats increased until stroke and then decreased. After 1 week, DR significantly increased systolic blood pressure compared to controls (200 ± 6 mmHg vs. 180 ± 4 mmHg, $P = 0.011$), possibly due to increased consumption of drinking water containing 1% NaCl, related to controls. However, this difference disappeared for 2 weeks (200 ± 8 mmHg vs. 197 ± 6 mmHg, $P = 0.795$). DR markedly delayed the onset of stroke compared to controls ($P = 0.022$) (Fig. 1B). Day of stroke (median) in the control rats was 34 days, whereas it was 70 days in DR rats. The survival rate followed the onset of stroke ($P = 0.014$) (Fig. 1C).

3.2. DR suppresses systemic inflammation

Plasma concentrations and mRNA expression levels in each tissue (liver, epididymal adipose tissue, spleen, and kidney) of major inflammatory cytokines (IL-1β, IL-6, TNF-α, and MCP-1) were measured after 2 weeks of DR, before any SHRSP showed signs of stroke. DR rats showed lower plasma concentrations of IL-1β, TNF-α, and MCP-1 than did control rats (although TNF-α did not reach significance, $P = 0.063$) (Fig. 2A). IL-6 was not detectable in either group.

Expression levels of IL-1β, IL-6, and TNF-α mRNA in liver were not different between control and DR rats. However, the expression level of MCP-1 mRNA was significantly lower in DR rats than in control rats (Fig. 2B). In epididymal adipose tissue, expression levels of IL-1β, IL-6, TNF-α, and MCP-1 mRNA were markedly lower in DR rats than in control rats (Fig. 2C). In spleen and kidney, only the expression level of MCP-1 mRNA in all tissues was lower in DR rats than in control rats, similar to that in liver (data not shown). These data suggested that decreases in plasma IL-1β, TNF-α, and MCP-1 concentrations in response to DR might be largely due to their altered expressions in adipose tissues and that DR suppressed systemic inflammation before the onset of stroke in SHRSP.

3.3. DR decreases adhesion molecule mRNA expression in CVECs and may suppress macrophage infiltration into brain

Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which are located on the surface of endothelial cells, bind to the leukocyte integrin and induce leukocyte transendothelial migration [18]. After 2 weeks of DR, expression levels of both ICAM-1 and VCAM-1 mRNA were lower in CVECs from DR rats compared to those in control rats (Fig. 2D). At the same time, macrophage infiltration was detected in several areas of the brain from control rats, but there were few macrophages in the same areas of the brain from DR rats

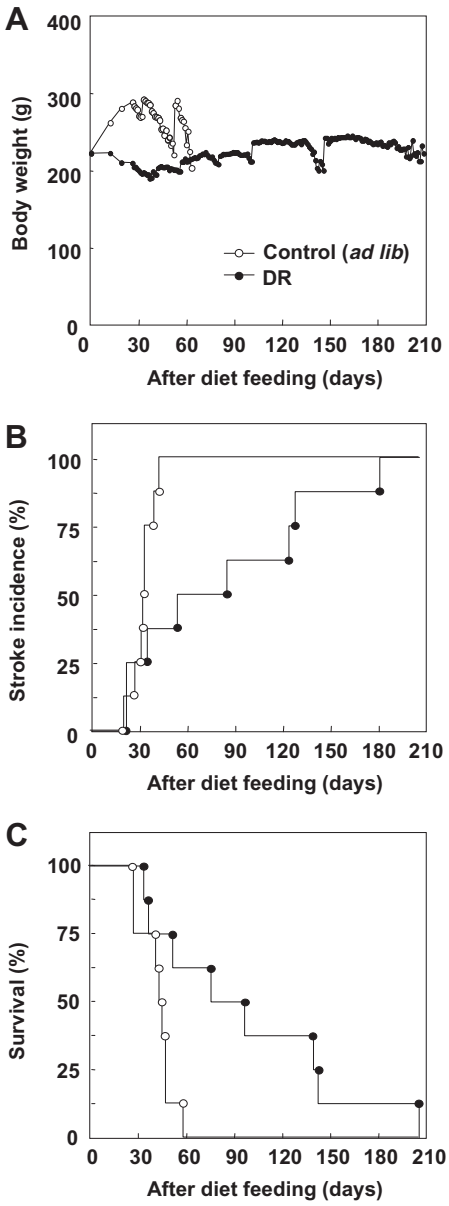

Fig. 1. Effect of DR on the onset of stroke and lifespan in SHRSP. (A) Body weights, (B) day of stroke onset, and (C) survival rate in control (*ad libitum*) and DR (dietary restriction) rats were determined as described in Section 2. Control, open circle; DR (rats consumed 50% of diet of control), closed circle. $n = 8$ in each group. $P = 0.022$ (stroke incidence); $P = 0.014$ (survival rate) vs. control in the log-rank test.

(Supplementary Fig. 1A). Quantitatively, although it was not significant (possibly due to the very low expression of CD68 mRNA [a macrophage marker] in whole brain), the expression level of CD68 mRNA in whole brain from DR rats was 20% lower than that in control rats (Supplementary Fig. 1B), suggesting that there is less macrophage infiltration into the brain in DR rats compared to control rats.

3.4. Inflammatory cytokines stimulate expression of adhesion molecules in CVECs

To examine whether inflammatory cytokines in blood could affect endothelial cells in the brain, CVECs from young SHRSP were exposed to inflammatory cytokines (IL-1β, IL-6, or TNF-α) for 72 h, and expression of their target genes (MCP-1, CRP, ICAM-1, and VCAM-1) was examined *in vitro*. IL-1β and TNF-α treatment

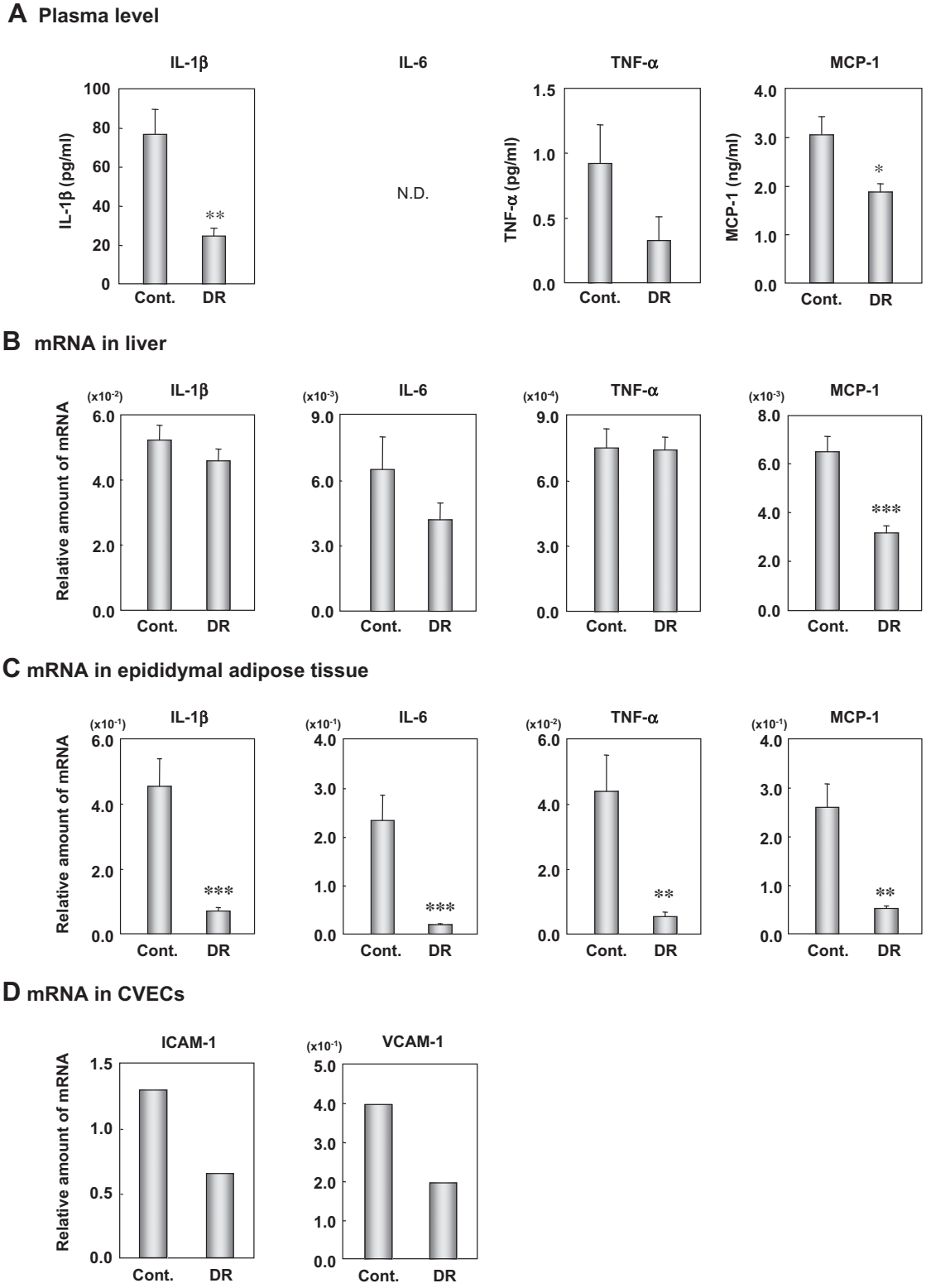

Fig. 2. Plasma inflammatory cytokine concentrations and mRNA expression levels related to cytokines in several tissues. (A) Plasma concentrations of IL-1β, IL-6, TNF-α, and MCP-1 in control and DR rats fed for 2 weeks were measured. Bars show SEM, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control rats by Student's *t*-test. $n = 5$ in each group. N.D.; non-detectable. (B, C) Inflammatory cytokine mRNA expression levels in liver and epididymal adipose tissue in control and DR rats fed for 2 weeks were measured by quantitative RT-PCR. Bars show SEM, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. control rats by Student's *t*-test. $n = 5$ in each group. (D) ICAM-1 and VCAM-1 mRNA expression levels in CVECs from control and DR rats were measured by quantitative RT-PCR. CVECs were isolated from brain and pooled among each group (five rats in control and four rats in DR group).

increased mRNA expression levels of MCP-1, CRP, ICAM-1, and VCAM-1 (Fig. 3A). IL-1β and TNF-α treatment also increased MCP-1 and CRP protein secretion into media and ICAM-1 protein in cells (Fig. 3B). However, IL-6 treatment did not affect expression of these inflammatory markers and adhesion molecules. These results indicate that inflammatory stimuli, IL-1β, and TNF-α,

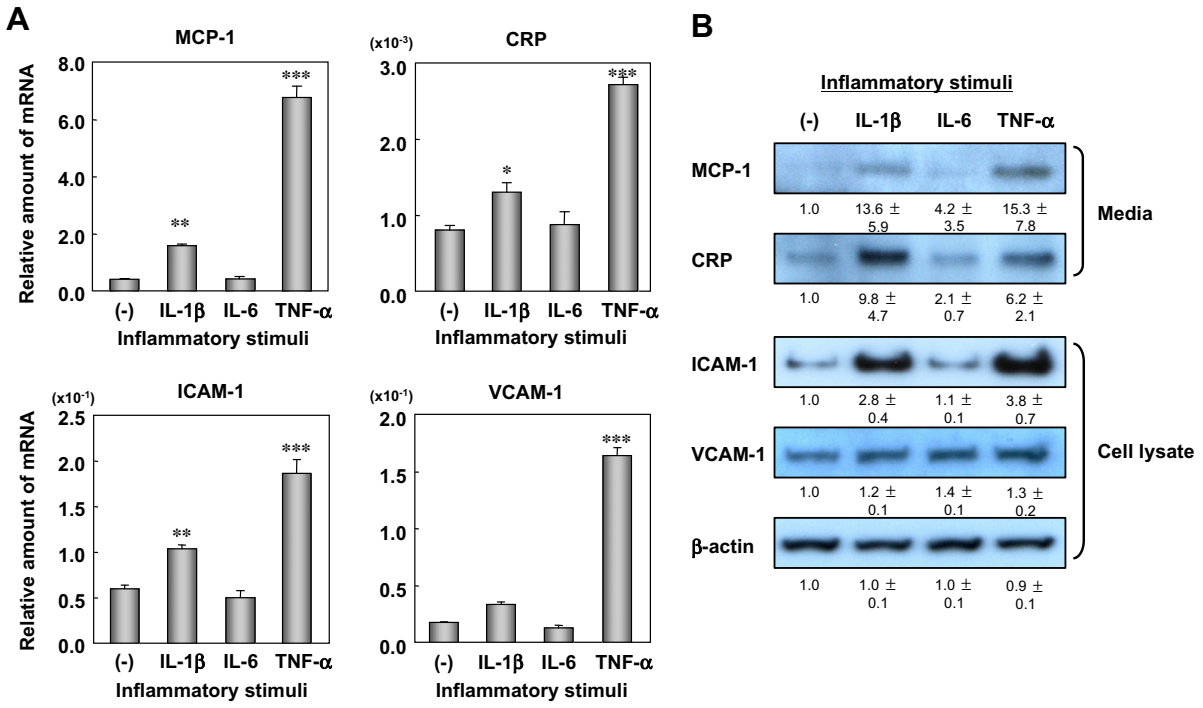

Fig. 3. Effect of inflammatory stimuli on expression of adhesion molecules in CVECs. (A) CVECs were treated with IL-1β, IL-6, or TNF-α (10 ng/ml) for 72 h. After incubation periods, total RNA was extracted for determination of mRNA expression levels by quantitative RT-PCR. Bars show SEM of triplicate determinations from one individual experiment, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. non-stimuli one-way ANOVA with a Bonferroni post hoc test. Two other experiments showed similar results. (B) Protein in the condition media and whole cell protein were extracted for Western blot analysis. Data are presented as mean ± SEM of densitometric ratios (non-stimuli was set as 1.0) of four independent experiments.

up-regulate chemokines and adhesion molecules in CVECs and may recruit blood leukocytes into the brain.

4. Discussion

DR markedly delayed the onset of stroke and extended the lifespan in SHRSP. This delay in stroke onset in response to DR might be mediated by suppression of inflammation. Increased inflammation in adipose tissue (or other tissues) in SHRSP led to increase in blood cytokine (IL-1β, TNF-α) concentrations. Blood IL-1β and TNF-α may increase expression of adhesion molecules in CVECs, by which blood leukocytes are recruited into the brain. IL-6 may not contribute to the brain inflammation in SHRSP because the plasma level of IL-6 was very low, and IL-6 treatment did not affect expression of inflammatory markers and adhesion molecules in CVECs.

Even though inflammation is recognized as a key event in stroke, it is controversial whether inflammation is the cause or result of stroke [19]. It was reported that SHRSP show increased systemic and local inflammation before the onset of stroke. Excess salt loading for 2 weeks increased ED-1 (=CD68)-positive cells (macrophage/activated microglia) in the brain before the onset of stroke [20]. Inflammatory marker proteins, such as transferrin, hemopexin, albumin in urine, or thiostatin in serum, were detected in salt-loaded SHRSP before the onset of stroke [21]. Acute-phase proteins were also detected in cerebrospinal fluid before there was any MRI evidence of brain abnormalities [22]. These data support the hypothesis that inflammation precedes stroke. In addition to animal studies, clinical studies already attended inflammatory events as targets of stroke therapy using statins. Meta-analysis of statin trials showed that statins might reduce the incidence of all strokes with no increase in hemorrhagic stroke [23]. Statins probably reduce stroke by a variety of mechanisms, including improving endothelial function and decreasing vascular inflammation [24], which we observed in DR SHRSP.

Despite having a lower body weight and less adipose tissue mass, a greater amount of inflammatory adipose tissue is present in SHRSP than in spontaneously hypertensive rats (SHR), in which stroke was not observed (unpublished observations, T. Chiba and O. Ezaki). However, DR effectively prevents the increased inflammation in adipose tissue observed in SHRSP; DR attenuated gene expression levels of inflammatory cytokines. It was reported that *ob/ob* mice on DR had longer lifespan than did *ad libitum* C57BL/6J mice, even though they still had greater amounts of adipose tissue than did the *ad libitum* C57BL/6J mice [25]. Thus, DR may decrease inflammation in adipose tissue, beyond its effect in decreasing adipose tissue mass.

Nuclear factor-kappa B (NF-κB) is a multi-subunit transcription factor and is involved in the induction of several inflammatory cytokines that are critically involved in the pathogenesis of chronic inflammatory diseases [26]. Lipopolysaccharide-induced NF-κB activity was significantly inhibited in splenocytes from energy-restricted mice [27]. Ten days of DR inhibited DNA binding activity of NF-κB in rat kidney [28]. In SHRSP, a diet containing broccoli sprouts high in glucoraphanin decreased NF-κB activation in kidney and tissue inflammation [29], and pioglitazone decreased left ventricular NF-κB binding activities and attenuated cardiac inflammation with little effect on blood pressure [30]. Therefore, DR might suppress NF-κB signaling and reduce the systemic inflammation in SHRSP independently of blood pressure.

Another possible mechanism by which DR delays the onset of stroke might be suppression of oxidative stress. Caloric restriction suppressed synthesis of reactive oxygen species (ROS) and increased expression of anti-oxidant enzymes, such as catalase, CuZn–SOD, and Mn–SOD [31]. Suppression of ROS by NADH oxidase inhibitor could suppress the onset of stroke in SHRSP [20]. However, significant differences were not detected in anti-oxidant enzyme mRNA expressions in CVECs between control and DR rats in our study (data not shown).

Malnutrition, and especially protein depletion, might aggravate cerebral hemorrhage. An inverse relation was also observed between animal protein intake and the risk of intraparenchymal hemorrhage in a human study [32]. In agreement with that human study, low-protein intake of less than 5% of total calories under *ad libitum* feeding conditions accelerated the onset of stroke in SHRSP [14]. DR rats in the present study had an absolute amount of protein equivalent to 10% of total calories under *ad libitum* feeding conditions. This suggests that DR without a marked decrease in protein intake might be effective in the prevention of stroke in humans.

5. Conclusions

DR suppressed systemic and local inflammation in SHRSP, and these effects may lead to a delay in the onset of stroke independent of any effect on blood pressure in SHRSP. A combination of DR and a hypotensive drug might be an effective method to prevent stroke in hypertensive subjects who have a chronic inflammatory state such as obesity.

Acknowledgments

The authors are thankful to Lisa Shiraishi for assistance with the care of the animals. This work was supported in part by a grant-in-aid for scientific research Kakenhi (20790199) from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT, Tokyo, Japan).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.07.048.

References

[1] G.J. Blake, P.M. Ridker, Inflammatory bio-markers and cardiovascular risk prediction, J. Intern. Med. 252 (2002) 283–294.
[2] P.M. Ridker, E. Danielson, F.A. Fonseca, J. Genest, A.M. Gotto Jr., J.J. Kastelein, W. Koenig, P. Libby, A.J. Lorenzatti, J.G. MacFadyen, B.G. Nordestgaard, J. Shepherd, J.T. Willerson, R.J. Glynn, Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein, N. Engl. J. Med. 359 (2008) 2195–2207.
[3] K. Okamoto, Y. Yamori, A. Nagaoka, Establishment of the stroke-prone spontaneously hypertensive rat, Circ. Res. 33/34 (1974) I143–I153.
[4] L. Sironi, E. Gianazza, P. Gelosa, U. Guerrini, E. Nobili, A. Gianella, B. Cremonesi, R. Paoletti, E. Tremoli, Rosuvastatin, but not simvastatin, provides end-organ protection in stroke-prone rats by antiinflammatory effects, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 598–603.
[5] R. Wilcox, M.G. Bousser, D.J. Betteridge, G. Schernthaner, V. Pirags, S. Kupfer, J. Dormandy, Effects of pioglitazone in patients with type 2 diabetes with or without previous stroke: results from PROactive (PROspective pioglitazone clinical trial in macroVascular events 04), Stroke 38 (2007) 865–873.
[6] T. Nakamura, E. Yamamoto, K. Kataoka, T. Yamashita, Y. Tokutomi, Y.F. Dong, S. Matsuba, H. Ogawa, S. Kim-Mitsuyama, Pioglitazone exerts protective effects against stroke in stroke-prone spontaneously hypertensive rats independently of blood pressure, Stroke 38 (2007) 3016–3022.
[7] T. Ishizuka, A. Niwa, M. Tabuchi, K. Ooshima, H. Higashino, Acetylsalicylic acid provides cerebrovascular protection from oxidant damage in salt-loaded stroke-prone rats, Life Sci. 82 (2008) 806–815.
[8] H.Y. Chung, M. Cesari, S. Anton, E. Marzetti, S. Giovannini, A.Y. Seo, C. Carter, B.P. Yu, C. Leeuwenburgh, Molecular inflammation: underpinnings of aging and age-related diseases, Ageing Res. Rev. 8 (2009) 18–30.
[9] R. Kalani, S. Judge, C. Carter, M. Pahor, C. Leeuwenburgh, Effects of caloric restriction and exercise on age-related, chronic inflammation assessed by C-reactive protein and interleukin-6, J. Gerontol. A Biol. Sci. Med. Sci. 61 (2006) 211–217.
[10] T. You, W.E. Sonntag, X. Leng, C.S. Carter, Lifelong caloric restriction and interleukin-6 secretion from adipose tissue: effects on physical performance decline in aged rats, J. Gerontol. A Biol. Sci. Med. Sci. 62 (2007) 1082–1087.
[11] J.O. Holloszy, L. Fontana, Caloric restriction in humans, Exp. Gerontol. 42 (2007) 709–712.
[12] A. Strom, R.A. Jensen, Mortality from circulatory diseases in Norway 1940–1945, Lancet 1 (1951) 126–129.
[13] M. Franco, P. Ordunez, B. Caballero, J.A. Tapia Granados, M. Lazo, J.L. Bernal, E. Guallar, R.S. Cooper, Impact of energy intake, physical activity, and population-wide weight loss on cardiovascular disease and diabetes mortality in Cuba, 1980–2005, Am. J. Epidemiol. 166 (2007) 1374–1380.
[14] T. Chiba, T. Itoh, M. Tabuchi, T. Satou, O. Ezaki, Dietary protein, but not carbohydrate, is a primary determinant of the onset of stroke in stroke-prone spontaneously hypertensive rats, Stroke 40 (2009) 2828–2835.
[15] P.D. Bowman, S.R. Ennis, K.E. Rarey, A.L. Betz, G.W. Goldstein, Brain microvessel endothelial cells in tissue culture: a model for study of blood–brain barrier permeability, Ann. Neurol. 14 (1983) 396–402.
[16] S.M. Hwang, S. Weiss, S. Segal, Uptake of L-[35S]cystine by isolated rat brain capillaries, J. Neurochem. 35 (1980) 417–424.
[17] S.K. Williams, J.F. Gillis, M.A. Matthews, R.C. Wagner, M.W. Bitensky, Isolation and characterization of brain endothelial cells: morphology and enzyme activity, J. Neurochem. 35 (1980) 374–381.
[18] E.S. Wittchen, Endothelial signaling in paracellular and transcellular leukocyte transmigration, Front. Biosci. 14 (2009) 2522–2545.
[19] P.J. Lindsberg, A.J. Grau, Inflammation and infections as risk factors for ischemic stroke, Stroke 34 (2003) 2518–2532.
[20] E. Yamamoto, N. Tamamaki, T. Nakamura, K. Kataoka, Y. Tokutomi, Y.F. Dong, M. Fukuda, S. Matsuba, H. Ogawa, S. Kim-Mitsuyama, Excess salt causes cerebral neuronal apoptosis and inflammation in stroke-prone hypertensive rats through angiotensin II-induced NADPH oxidase activation, Stroke 39 (2008) 3049–3056.
[21] L. Sironi, E. Tremoli, I. Miller, U. Guerrini, A.M. Calvio, I. Eberini, M. Gemeiner, M. Asdente, R. Paoletti, E. Gianazza, Acute-phase proteins before cerebral ischemia in stroke-prone rats: identification by proteomics, Stroke 32 (2001) 753–760.
[22] L. Sironi, U. Guerrini, E. Tremoli, I. Miller, P. Gelosa, A. Lascialfari, I. Zucca, I. Eberini, M. Gemeiner, R. Paoletti, E. Gianazza, Analysis of pathological events at the onset of brain damage in stroke-prone rats: a proteomics and magnetic resonance imaging approach, J. Neurosci. Res. 78 (2004) 115–122.
[23] P. Amarenco, J. Labreuche, P. Lavallee, P.J. Touboul, Statins in stroke prevention and carotid atherosclerosis: systematic review and up-to-date meta-analysis, Stroke 35 (2004) 2902–2909.
[24] J.K. Liao, Beyond lipid lowering: the role of statins in vascular protection, Int. J. Cardiol. 86 (2002) 5–18.
[25] D.E. Harrison, J.R. Archer, C.M. Astle, Effects of food restriction on aging: separation of food intake and adiposity, Proc. Natl. Acad. Sci. USA 81 (1984) 1835–1838.
[26] Q. Li, I.M. Verma, NF-kappa B regulation in the immune system, Nat. Rev. Immunol. 2 (2002) 725–734.
[27] A. Bhattacharya, B. Chandrasekar, M.M. Rahman, J. Banu, J.X. Kang, G. Fernandes, Inhibition of inflammatory response in transgenic fat-1 mice on a calorie-restricted diet, Biochem. Biophys. Res. Commun. 349 (2006) 925–930.
[28] K.J. Jung, E.K. Lee, J.Y. Kim, Y. Zou, B. Sung, H.S. Heo, M.K. Kim, J. Lee, N.D. Kim, B.P. Yu, H.Y. Chung, Effect of short term calorie restriction on pro-inflammatory NF-kB and AP-1 in aged rat kidney, Inflamm. Res. 58 (2009) 143–150.
[29] M.H. Noyan-Ashraf, L. Wu, R. Wang, B.H. Juurlink, Dietary approaches to positively influence fetal determinants of adult health, FASEB J. 20 (2006) 371–373.
[30] Q.N. Diep, F. Amiri, K. Benkirane, P. Paradis, E.L. Schiffrin, Long-term effects of the PPAR gamma activator pioglitazone on cardiac inflammation in stroke-prone spontaneously hypertensive rats, Can. J. Physiol. Pharmacol. 82 (2004) 976–985.
[31] S. Agarwal, S. Sharma, V. Agrawal, N. Roy, Caloric restriction augments ROS defense in *S. cerevisiae*, by a Sir2p independent mechanism, Free Radic. Res. 39 (2005) 55–62.
[32] H. Iso, S. Sato, A. Kitamura, Y. Naito, T. Shimamoto, Y. Komachi, Fat and protein intakes and risk of intraparenchymal hemorrhage among middle-aged Japanese, Am. J. Epidemiol. 157 (2003) 32–39.