

Original Article

Steroid pulse therapy impaired endothelial function while increasing plasma high molecule adiponectin concentration in patients with IgA nephropathy

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Abstract

Background. Decreased plasma adiponectin is associated with impaired endothelial function and, thereby, increased risk for cardiovascular events. Glucocorticoid (GC) affects vascular endothelial cells either favourably or harmfully depending upon the dosages and duration. We examined the effect of GC pulse therapy on vascular endothelial function.

Methods. Fourteen young patients with IgA nephropathy were evaluated for flow-mediated vasodilation (FMD), plasma levels of adiponectin both in high molecular weight (HMW adiponectin) form and in single molecular form (total adiponectin), hepatocyte growth factor (HGF), asymmetric dimethylarginine (ADMA), and high-sensitive C-reactive protein, before and after a course of GC pulse therapy.

Results. GC pulse therapy significantly decreased FMD (from 7.2 ± 2.6 to $5.7 \pm 2.5\%$, $P < 0.01$). Meanwhile, plasma adiponectin levels were significantly augmented (total adiponectin: from 10.2 ± 4.0 to $12.1 \pm 6.3 \mu\text{g/ml}$, $P < 0.05$; HMW: from 6.5 ± 3.2 to $7.7 \pm 3.3 \mu\text{g/ml}$, $P < 0.05$). In parallel, elevated concentrations of serum HGF (from 0.28 ± 0.12 to $0.63 \pm 0.38 \text{ ng/ml}$, $P < 0.01$) and plasma ADMA (from 0.45 ± 0.07 to $0.53 \pm 0.04 \text{ nmol/ml}$, $P < 0.05$) were observed.

Conclusions. GC pulse therapy impaired endothelial function while increasing plasma adiponectin levels, which may in turn restore the endothelial function in patients with IgA nephropathy.

Keywords: asymmetric dimethylarginine; endothelial dysfunction; glucocorticoid; hepatocyte growth factor; high molecular weight adiponectin; IgA nephropathy

Introduction

Endothelial dysfunction is associated, not only with classic cardiovascular risk factors including advanced age, male gender, dyslipidaemia, cigarette smoking, hypertension and diabetes mellitus [1], but also with novel risk factors such as chronic kidney disease [2,3] and hypoadiponectinaemia [4]. Among various measures for endothelial function, brachial artery flow-mediated vasodilation (FMD) assessed by high-frequency ultrasound, which is an established non-invasive technique, correlates with coronary artery FMD, and has been shown to predict long-term cardiovascular events [5,6].

Plasma levels of adiponectin, an adipose-derived hormone, decreases in obese subjects, and hypoadiponectinaemia are associated with ischaemic heart disease. The adenovirus-mediated adiponectin migrated to foam cells in the fatty streak lesions and reduced atherosclerosis in apolipoprotein E-deficient mice [7]. The high molecular weight (HMW) form of adiponectin, but not the low molecular form has been shown to suppress endothelial cell apoptosis *in vitro*, and is suggested to specifically confer vascular-protective activities [8]. Thus, adiponectin is an important target molecule in the prevention of cardiovascular diseases.

High-dose glucocorticoid (GC) exerts cardiovascular and neuronal protection through a non-transcriptional activation of endothelial nitric oxide synthase (eNOS) in mice [9]. In contrast, GC excess induces superoxide production in vascular endothelial cells and elicits vascular endothelial dysfunction in patients with

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various autoimmune disorders [10]. It has recently been reported that short-term intervention with 3 mg dexamethasone twice daily for 5 days decreased nitroglycerin-mediated vasodilatation (NTD), without affecting FMD in healthy volunteers [11]. Thus, GC excess may affect endothelial function either favourably or harmfully depending on the way it is used.

In the treatment of IgA nephropathy (IgAN), GC pulse therapy combined with or without tonsillectomy is effective not only in reducing proteinuria but also in inhibiting renal function deterioration [12,13]. At the conception of chronic kidney disease, remission or regression of IgAN may serve as a risk reduction for future coronary events; however, the impact of GC pulse therapy on endothelial function must be assured particularly because the majority of patients with IgAN are young and free from classic cardiovascular risk factors. In the present study, we examined the effect of GC pulse therapy on vascular endothelial function in patients with IgAN.

Methods

Subjects

This study was approved by the local Ethics Committee. All patients signed an informed written consent form to participate.

Fourteen patients aged 23–41 years were recruited from renal clinics of Okayama University from April 2004 to March 2005. All patients had been diagnosed with IgAN by percutaneous renal biopsy, and underwent tonsillectomy at least 2 weeks prior to receiving three courses of GC pulse therapy (one course of GC pulse regimen consists of methylprednisolone 500 mg/day for 3 consecutive days). None of the participants had a history of cardiovascular diseases or a history of cigarette smoking. Blood sampling and vascular studies were performed before and after the first course of GC pulse therapy.

Vascular studies

Studies were performed in patients after resting supine for at least 15 min in a quiet, temperature controlled room (20–25°C), between 3:00 p.m. and 5:00 p.m. at Okayama University Hospital. Flow-mediated vasodilation (FMD) and nitroglycerin-induced vasodilation (NTD) of the brachial artery was assessed using high resolution ultrasonography with a 7.5 MHz linear transducer (ProSound, SSD-3500, ALOKA CO., LTD), as previously described [5]. Briefly, the right brachial artery images were acquired above the antecubital fossa in the longitudinal plane of the artery. FMD was induced by inflating a blood pressure cuff around the forearm to 200 mmHg for 5 min and then deflating the cuff. The diameter of the right brachial artery was assessed 60–90 s after deflation of the cuff. Thereafter, a 15-min period was allowed for recovery of the vessel, after which a second baseline image of the brachial artery was obtained. A sublingual dose of nitroglycerin tablet (0.4 mg) was administered, and NTD was assessed by imaging the artery continuously for 4 min. The images for measurement of the diameter were obtained between the 3rd and 4th min.

An average of three measurements of the brachial artery diameter was used. All measurements were performed by two experienced investigators.

Biomarkers study

Serum total adiponectin was measured by enzyme-linked immunosorbent assay using human adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo). Plasma asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) concentrations were measured by high-performance liquid chromatography with pre-column derivatization with *o*-phthalaldehyde (SRL, Inc., Tokyo). Plasma levels of high-sensitive C-reactive protein (hsCRP) were assessed with a validated high-sensitivity assay, particle-enhanced immunonephelometry using the BN Systems (SRL, Inc., Tokyo). The plasma was HMW form of adiponectin and serum hepatocyte growth factor (HGF) were evaluated by enzyme-linked immunosorbent assay (SRL, Inc., Tokyo).

Statistics

Changes in the values before and after GC pulse therapy were compared. The results are presented as the mean \pm SD. Significant difference is determined by Student's paired *t*-test or Wilcoxon's rank test, where appropriate. A value of $P < 0.05$ was considered to be statistically significant.

Results

Patients' characteristics

Subject characteristics including both before and after the steroid pulse therapy, are shown in Table 1. Blood pressure, renal function and serum lipids were not impaired in any subject before the treatment.

Table 1. Patients' characteristics

Parameters	Before	After	<i>P</i> -value
Gender (M/F), <i>n</i>	6/8		
Age (years)	31.6 \pm 7.8		
BW (kg)	58.7 \pm 10.0	59.6 \pm 11.0	0.7795
BUN (mg/dl)	14.1 \pm 4.5	16.4 \pm 4.8	0.0543
Cr (mg/dl)	0.87 \pm 0.22	0.91 \pm 0.22	0.1069
U.A (mg/dl)	5.8 \pm 1.2	5.4 \pm 1.6	0.1301
CCr (ml/min)	81.8 \pm 16.8	75.7 \pm 17.0	0.6690
T.P (g/dl)	6.9 \pm 0.3	6.5 \pm 0.6	<0.0001
Alb (g/dl)	3.9 \pm 0.1	3.7 \pm 0.2	0.0002
T.Cho (mg/dl)	202 \pm 29	206 \pm 33	0.6177
LDL-C (mg/dl)	120 \pm 24	113 \pm 24	0.3765
SBP (mmHg)	108 \pm 11	113 \pm 9	0.0739
DBP (mmHg)	74 \pm 8	69 \pm 11	0.0991
BS (mg/dl)	93 \pm 12	101 \pm 24	0.2288
U-TP (g/day)	0.680 \pm 0.475	0.476 \pm 0.402	0.0052

BW, body weight; BUN, blood urea nitrogen; Cr, creatinine; U.A, uric acid; CCr, creatinine clearance; T.P, total protein; Alb, albumin; T.Cho, total cholesterol; LDL-C, low density lipid cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; BS, blood sugar; U-TP, urine protein; data are presented mean \pm S.D.

Serum total protein, albumin and urine protein were significantly decreased after the therapy. Plasma low-density lipoid cholesterol (LDL-C) also dropped after the treatment, but not significantly. Body weight, total cholesterol, systolic blood pressure and blood sugar were slightly enhanced after the treatment, but not significantly. Three patients were given angiotensin II type 1 receptor antagonist (losartan, 25 mg/day), the dosage of which was unchanged throughout the study. No patient was prescribed a statin or vitamin E.

FMD and NTD findings

FMD after GC pulse therapy was decreased significantly compared with baseline FMD (baseline, $7.2 \pm 2.6\%$; after treatment, $5.7 \pm 2.5\%$; $P < 0.01$) (Figure 1). Meanwhile, NTD values did not change significantly between before and after GC treatment.

Changes of cardiovascular biomarkers for endothelial functions

Serum HMW adiponectin, total adiponectin, ADMA and HGF after the therapy were significantly increased compared with respective baseline (HMW adiponectin, baseline, $6.5 \pm 3.2 \mu\text{g/ml}$; after treatment, $7.7 \pm 3.3 \mu\text{g/ml}$; $P < 0.05$, total adiponectin, baseline, $10.2 \pm 4.0 \mu\text{g/ml}$; after treatment, $12.1 \pm 6.3 \mu\text{g/ml}$; $P < 0.05$, ADMA, baseline, $0.45 \pm 0.07 \text{ nmol/ml}$; after treatment, $0.53 \pm 0.04 \text{ nmol/ml}$; $P < 0.05$, HGF, baseline, $0.28 \pm 0.12 \text{ ng/ml}$; after treatment, $0.63 \pm 0.38 \text{ ng/ml}$; $P < 0.01$, respectively) (Figures 2A,B and 3A,C). Serum SDMA did not change between before

and after the treatment (baseline, $0.53 \pm 0.18 \text{ nmol/ml}$; after treatment, $0.54 \pm 0.26 \text{ nmol/ml}$; $P = 0.65$) (Figure 3B). Interestingly, plasma HMW adiponectin concentration in men after the therapy rose significantly (from 5.8 ± 2.4 to $7.8 \pm 3.5 \mu\text{g/ml}$, $P < 0.05$), whereas in women there was no significant rise (from 7.3 ± 3.7 to $7.5 \pm 3.3 \mu\text{g/ml}$) (Figure 2C). Serum hsCRP after the therapy was significantly decreased compared with baseline (baseline, $640 \pm 460 \text{ ng/ml}$; after treatment, $178 \pm 133 \text{ ng/ml}$; $P < 0.01$) (Figure 3D). There were no statistical relations among the changes in FMD, plasma HMW adiponectin and HGF each other.

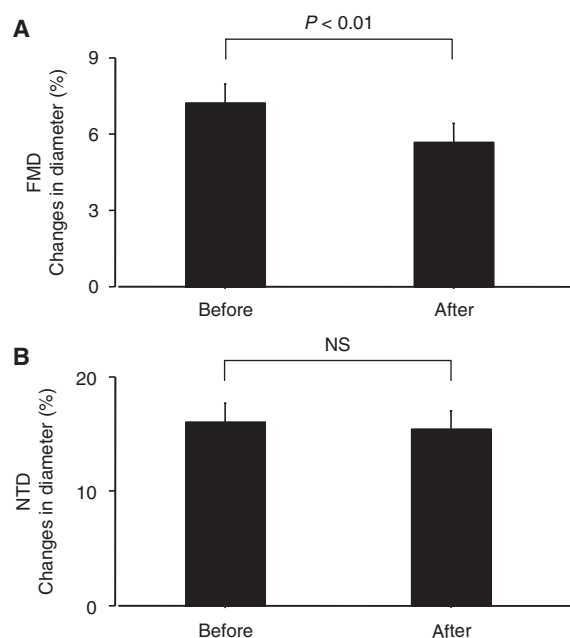


Fig. 1. Percentage change of brachial artery before and after GC pulse treatment. (A) Flow-mediated endothelium-dependent vasodilation (FMD). (B) Endothelium-independent, nitro-induced vasodilation (NTD). Values are presented as the mean \pm SEM.

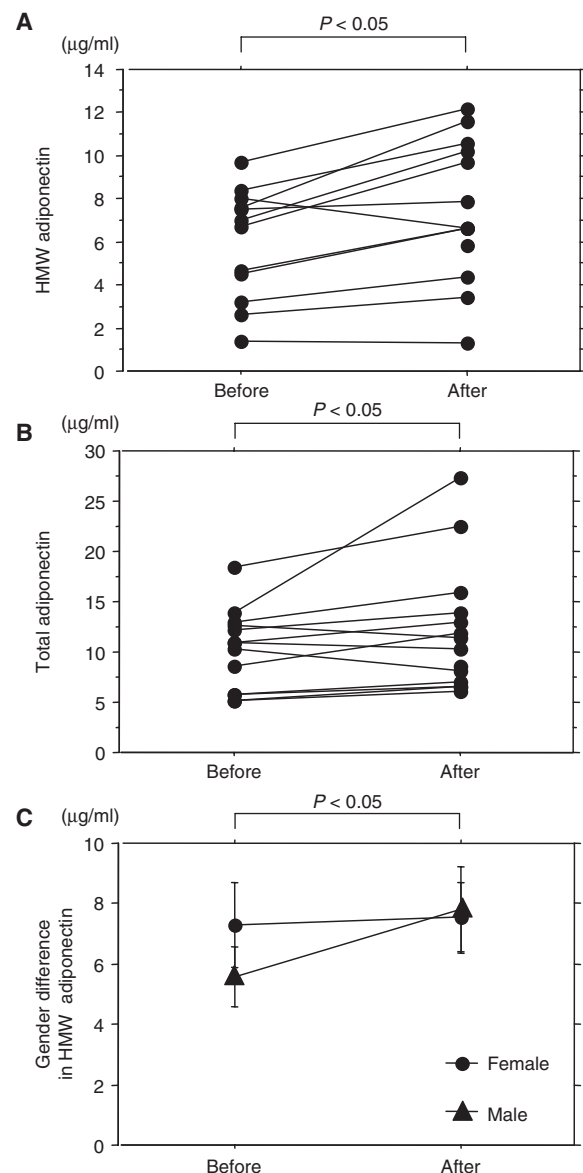


Fig. 2. (A) Value of serum high molecular weight (HMW) form of adiponectin before and after GC pulse treatment. (B) Value of serum total adiponectin before and after GC pulse treatment. (C) Value of HMW adiponectin in each gender before and after GC pulse treatment. Values are presented as the mean \pm SEM.

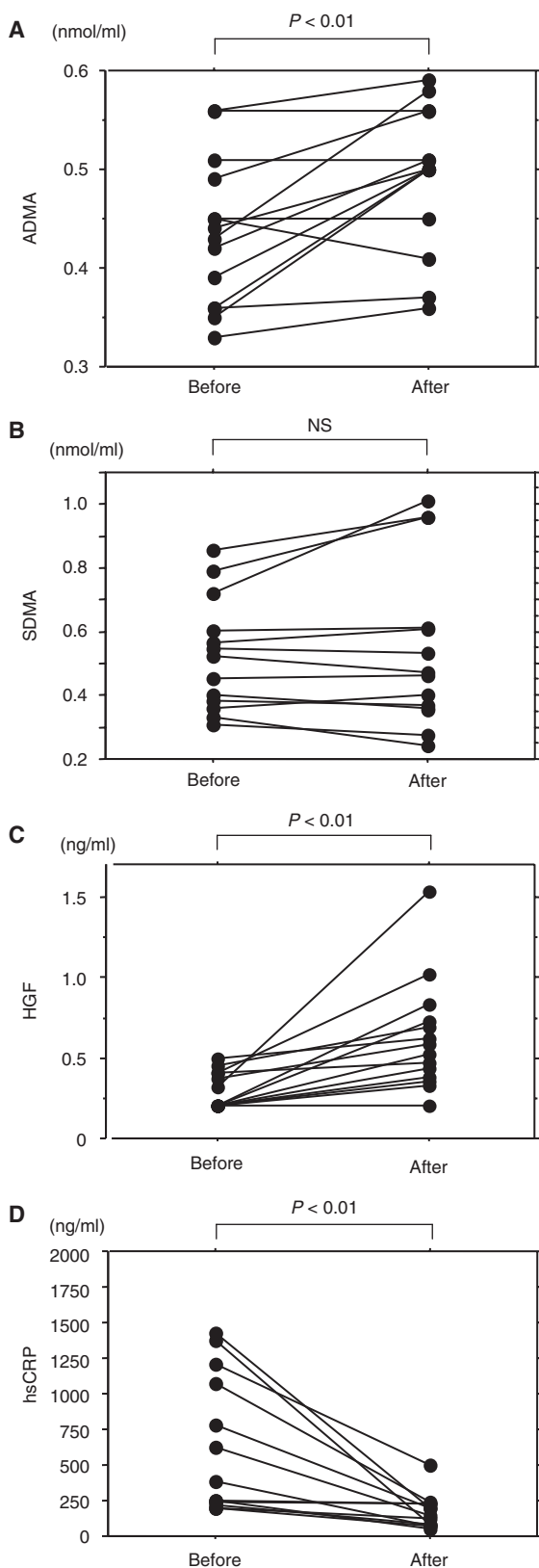


Fig. 3. (A) Value of asymmetric dimethylarginine (ADMA) before and after GC pulse treatment. (B) Value of symmetric dimethylarginine (SDMA) before and after GC pulse treatment. (C) Value of hepatocyte growth factor (HGF) before and after GC pulse treatment. (D) Value of serum high-sensitive C-reactive protein (hsCRP) before and after GC pulse treatment.

Discussion

To our knowledge, this is the first study to examine the effects of steroid pulse therapy on vascular function in patients with IgAN. We demonstrated that GC pulse therapy significantly attenuates FMD and simultaneously affects plasma levels of adiponectin, HGF and ADMA in the short term. All subjects were relatively young, normotensive, and had haematuria and proteinuria, but not dyslipidaemia, diabetes mellitus or cigarette smoking habit. Plasma LDL-C, body weight, total cholesterol, systolic blood pressure and blood sugar, which are established factors that alter FMD, were changed after the therapy, but not significantly. This suggests that these factors did not contribute mainly to decreasing FMD in this population. Brotman [11] reported that short-term exposure to dexamethasone did not attenuate FMD in healthy non-smoking men, in contrast to our finding that methylpredonisolone decreased FMD significantly in patients with IgAN. The marked difference between the two studies is the existence of IgAN, and proteinuria in particular. Our patients showed relatively low FMD at baseline in accordance with the previous reports in patients with IgAN [3] and with proteinuria ranging from nephrosis to micro-albuminuria [14]. This suggests that impaired endothelial function might be more sensitive to GC excess, although identifying the underlying mechanisms is beyond the scope of this study.

Iuchi *et al.* [10] demonstrated that GC excess, orally administered prednisolone in the long-term, impaired endothelial-dependent forearm blood flow probably through the perturbation of nitric oxide (NO) availability by overproduction of reactive oxygen species (ROS) in the vasculature. On the other hand, intraperitoneally applied high-dose dexamethasone was demonstrated to increase blood flow via non-transcriptional activation of eNOS in mice [9]. Our finding of methylpredonisolone pulse-induced significant reduction in FMD is in agreement with the former report. In this study, at first, baseline ADMA concentration of those with IgAN was significantly higher than that of age-matched healthy volunteers (IgAN: 0.430 ± 0.06 nmol/ml, control: 0.374 ± 0.05 nmol/ml, $P < 0.05$). Then, there was a significant increase in plasma ADMA and no significant change in serum SDMA between before and after the pulse therapy. ADMA is partly metabolized by dimethylarginine dimethylaminohydrolase (DDAH). In contrast, SDMA is not enzymatically metabolized by DDAH. Therefore, elevated plasma ADMA, an endogenous inhibitor of eNOS, following GC pulse therapy can indicate the overproduction of ROS in our study. Antioxidant conditions in the vasculature may determine the endothelium response to GC-induced oxidative stress regarding whether to tolerate or not.

In the present study, we found that circulating adiponectin levels were significantly augmented after GC pulse therapy in both HMW form and single form.

It is surprising because others have demonstrated negative regulation of adiponectin by dexamethasone not only at the gene expression level in 3T3-L1 adipocytes [15] and human visceral adipose tissue [16], but also at the secretion level from human adipocytes. Anti-inflammatory action of GC pulse therapy as shown in the decrease in hsCRP might partly explain the elevation of plasma adiponectin levels, since interleukin-6 (IL-6) is a potent inhibitor of adiponectin at both the transcription and the secretion level in 3T3-L1 adipocytes [17]. However, these findings are provided only *in vitro*. There can be more relevant determinants for plasma adiponectin levels *in vivo*, since there was no direct influence of cortisol levels on plasma adiponectin levels in patients with surgically cured Cushing's disease [18].

Interestingly, there tends to be a gender difference, even though it is statistically not significant, in plasma HMW adiponectin levels at baseline between men ($5.8 \pm 2.4 \mu\text{g/ml}$) and women ($7.3 \pm 3.7 \mu\text{g/ml}$) in the present study. Selective inhibition of HMW adiponectin secretion by testosterone has been demonstrated in rats [19]. However, the gender difference was abolished unexpectedly by GC pulse therapy in this study (Figure 2C). The underlying mechanisms are unknown.

Endothelial dysfunction is an early and modifiable step in the atherogenesis [1]. Adiponectin possesses anti-inflammatory and anti-atherogenic properties [7,8]. Therefore increased plasma adiponectin may affect endothelial cells favourably, that is, restore endothelial function following GC pulse therapy. Under the restriction of an IgAN clinical pass, we could not include follow-up evaluation of FMD in this study. However, we performed repeated FMD measurements in patients receiving steroid pulse therapy with various autoimmune diseases, and surmise that GC-pulse-induced FMD-reduction is completely recovered within 2–3 weeks (data not shown). In fact, one patient from this study has a fully recovered FMD in a later examination (data not shown). We hypothesize that GC-pulse-induced adiponectin, HMW form in particular, may play important roles in restoring endothelial dysfunction. In other words, plasma adiponectin may be up-regulated as a counter regulation to the endothelial damages incurred by GC pulse therapy. A similar finding is documented in the regulation of plasma HGF, where 'injurin' locally produced factors in response to cell damage mediate plasma HGF increase from a remote organ, which in turn can protect and/or repair the endothelial cells. Thus, elevated plasma HGF levels after the GC treatment may indicate endothelial cell injury, and lead to endothelial cell repair [20]. There was no statistical relation between the changes in plasma HMW adiponectin and HGF. This suggests that the changes in these hormones through the therapy were independent of each other, indicating that both could contribute to restoring endothelial dysfunction by different ways. However, we cannot verify that

hypothesis in this study design. Further study is needed.

In this study, GC pulse therapy induced endothelial dysfunction, whereas it increased plasma levels of HMW and total adiponectin as well as HGF. These findings are limited to patients with IgAN, therefore further studies are required to apply to other diseases. Raised adiponectin and HGF may in turn restore endothelial function; however, the impact of them on kidney function requires further investigation. Generally concerned GC's adverse effects arise during long-term use, including hypertension, dyslipidaemia and diabetes mellitus. Compared with such proatherogenic effects, transient endothelial dysfunction induced by pulse therapy seems acceptable. Finally, GC pulse therapy can be performed safely in patients with IgAN. Further study is required to investigate the influence of GC pulse therapy on endothelial function in larger study populations with other diseases.

Conflict of interest statement. None declared.

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