Original Articles

Effect of increased levels of adiponectin by administration of the adenovector rAAV2/1-Acrp30 on glucose, lipid metabolism and ultrastructure of the aorta in Goto–Kakizaki rats with arteriosclerosis

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ABSTRACT

Background. We used recombinant adeno-associated virus vector of adiponectin (AAV2/1-Acrp30) to study the effects of increased levels of adioponectin (by the administration of rAAV2/1-Acrp30) on arteriosclerosis, glucose and lipid metabolism in Goto–Kakizaki (GK) rats with arteriosclerosis.

Methods. Thirty GK rats with arteriosclerosis were divided into 3 equal groups: control group 1, control group 2 and the rAAV2/1-Acrp30-administered group. Saline, virus vector or rAAV2/1-Acrp30 (10^{12} ng/ml) vector genomes administered to the rats in the corresponding group by intramuscular injection to the posterior limb by single administration, respectively. After 8 weeks, fasting blood glucose, 2-hour postprandial blood glucose, glycosylated haemoglobin, serum insulin, serum total cholesterol, triglycerides, high-density lipoprotein and low-density lipoprotein were measured in each group, and the ultrastructure of the aorta was seen by light and electron microscopy.

Results. Compared with control groups 1 and 2, in the rAAV2/1-Acrp30 group, there was a decrease in urine volume, fasting blood glucose, 2-hour postprandial blood glucose, glycosylated haemoglobin, serum total cholesterol, triglycerides and low-density lipoprotein, and an increase in body weight and high-density lipoprotein ($p<0.05$), while the level of serum insulin was not changed ($p>0.05$). Ultrastructure studies of the aorta showed that aortosclerosis in the rAAV2/1-Acrp30-administered group was less, and fewer lipid droplet vacuoles were seen in the vascular endothelial cytoplasm. Also various cell organelles and internal elastic lamina were seen, and there was no formation of lipid droplet and foam cells in the cytoplasm of the media of the smooth muscle.

Conclusion. Adiponectin could improve blood glucose and lipid parameters and decrease atherosclerosis in the aorta of GK rats.

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INTRODUCTION

Disturbance of lipid metabolism is an important initiating event of atherosclerosis. Adiponectin is secreted mainly by adipose tissue and takes part in regulating lipid metabolism, and anti-inflammatory and anti-atherosclerotic events. It correlates independently with serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL).[1] Studies[2,3] have shown that decreased level of adiponectin is an independent risk factor for diabetes and its complications. Any intervention that increased or normalized its level could retard the development of diabetic macroangiopathy. Goto–Kakizaki (GK) rats develop insulin resistance and show slow response to insulin in the transformation of glucose in hepatocytes and skeletal muscle cells, along with an increase in blood glucose and lipid disorders.[4,5] We had previously constructed a recombinant adeno-associated virus (AAV) vector expressing adiponectin (AAV2/1-Acrp30).[5] In this study, we administered adiponectin through the virus rAAV2/1-Acrp30 to GK rats with arteriosclerosis, and investigated its effects on glucose and lipid metabolism and the ultrastructure of the aorta.

METHODS

This investigation abides by the Guidelines of the Care and Use of Laboratory Animals issued by the Chinese Council on Animal Research and the Guidelines of Animal Care. Six-week-old male GK rats (weighing 350–400 g) were obtained from Shanghai Slac Laboratory Animal Co. Ltd. The rats were maintained at the Animal Center of Xiangya Hospital Central-South University in an ambient temperature of 22±1 °C, relative humidity of 59%–61% and normal photoperiod of 12 hours of light and 12 hours darkness. We have previously established an animal model of atherosclerosis in type 2 diabetic rats.[6] The rats were fed a high fat diet (normal diet 87.5%, refined lard 10%, cholesterol 12%, bile salt 0.3% and propylthiouracil 0.2%) and administered Nω-nitio-L-arginine methyl ester (L-NAME) intragastrically for 8 weeks.

AAV2/1-Acrp30 construction

Recombinant AAV vectors expressing adiponectin were generated, purified and titred at Central South University Cancer Core Laboratory as previously described.[4]

Animal groups and treatment

Thirty GK rats with arteriosclerosis were divided into three groups. The two control groups received saline and the virus vector, respectively, while the third group received rAAV2/1-Acrp30 (10^{12} ng/ml) by intramuscular injection in the posterior limbs.

The volume of drinking water, diet, activity, urine volume and body weight of the rats was recorded daily for 8 weeks.

Sample collection

During the experiment, 4 ml blood was obtained from the orbital vein of each rat to measure fasting blood sugar, TC, TG, LDL and HDL. A glucose solution (200 g/L) was given via a feeding tube at a dose of 2 g/kg body weight and 2 ml blood was collected 2 hours after its administration[7] at 0, 4 and 8 weeks. After 8 weeks, the rats were weighed after being anaesthetized. The aorta was harvested for histopathological analysis and a part of the sample was fixed in 10% neutral buffered formalin overnight and embedded in paraffin. Paraffin sections (5 μm thick) were stained with haematoxylin and eosin. Others were fixed in 25% glutaraldehyde for electron microscopy.

Blood lipid profiles, glucose and insulin

TC, TG, LDL, HDL and glycosylated haemoglobin were assayed by HITACH 717 fully automatic biochemical instrument (Hitach Electrical Co. Ltd). Blood glucose was measured with a portable glucose meter (ACCU-CHEK®Advantage, Roche, Switzerland). Plasma insulin was measured by insulin-specific radioimmuno-assay kits (Millipore, USA).

Exogenous adiponectin mRNA expression in the aorta

Total RNA was prepared with an RNeasy Midi Kit (Qiagen, Tokyo, Japan) from the aorta. Reverse transcriptase–polymerase chain reaction (RT-PCR) was done by a GeneAmp 5700 and a TaqMan One-Step RT-PCR Master Mix Reagents Kit (Applied Biosystems, Foster City, USA) according to the manufacturer's instruction.

Expression values for each gene were normalized to 18 S ribosomal RNA (TaqMan ribosomal RNA control reagents; Applied Biosystems). The primers and TaqMan probes used in these experiments were as follows: exogenous adiponectin (450bp) sense-primer: 5′-TAGAAGGCACAGTCGAGG-3′; antisense-primer: 5′-GGAACT TGGGGACAGTGAC-3′; β-actin (214bp) sense-primer: 5′-ATGGTGGGTATGGGTCAGAA-3′; antisense-primer: 5′-TGGCCTTAGGGTTCAGAGG-3′.

Statistical analysis

The differences between groups were evaluated by ANOVA followed by independent *t* test. All data were expressed as mean (SD) and all analyses were done using SPSS 11.0. A p value <0.05 was considered statistically significant.

RESULTS

General condition of GK rats with arteriosclerosis

GK rats had increased blood glucose, polyphagia, polyuria and polydipsia. In the control groups, 8 rats suffered from cataract after 20 days, 1 developed diarrhoea repeatedly and 2 rats in each group died of congestive heart failure. In the rAAV2/1-Acrp30-administered group, only 2 rats had cataract and 1 rat died of repeated diarrhoea and hypoglycaemia. The rest had good health without dermal erosion or any stool abnormality.

Effect of rAAV2/1-Acrp30 on glucose and lipid metabolism and body weight

Compared to the control groups, in the rAAV2/1-Acrp30-treated rats at 4 and 8 weeks TG, TC, LDL, urine volume, fasting and 2 hours postprandial blood sugars and glycosylated haemoglobin were lower ($p<0.05$). However, HDL increased ($p<0.05$) but insulin level showed no difference ($p<0.05$). The body weight of the rats in the control groups increased slowly ($p<0.05$). In the rAAV2/1-Acrp30 group, the changes at 4 and 8 weeks post-administration were similar and there was no difference in HDL ($p>0.05$; Tables I and II).

Effect of rAAV2/1-Acrp30 on ectogenic adiponectin mRNA in the aorta

Exogenous adiponectin mRNA was demonstrable in the aorta of the rAAV2/1-Acrp30-administered group but not in the control groups. This suggested that rAAV2/1-Acrp30 was successfully transfected into the aorta (Table I, Fig. 1)

TABLE I. Changes of grey scale value of Acrp30/β-actin mRNA in different groups

Group	*n*	Mean (SD) Acrp30/β-actin
Control 1 (saline)	8	0.00 (0.00)
Control 2 (control virus)	8	0.00 (0.00)
rAAV2/1-Acrp30-administered	9	0.47 (0.04)*

* $p<0.05$, rAAV2/1-Acrp30-administered group *v.* control groups

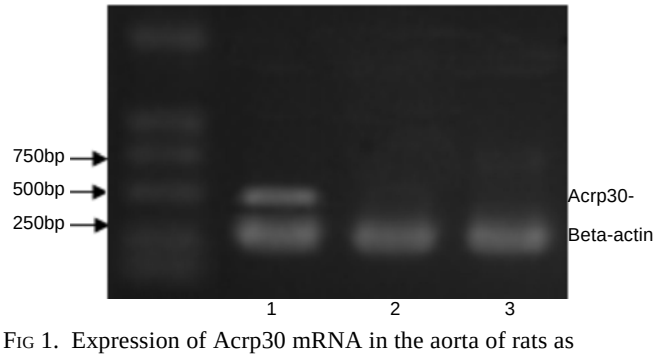

FIG 1. Expression of Acrp30 mRNA in the aorta of rats as determined by reverse transcriptase-polymerase chain reaction. Lane 1. rAAV2/1-Acrp30-administered group. Lane 2 Control group 1 (saline). Lane 3. Control group 2 (control virus)

TABLE II. Changes in various parameters in the different groups of rats

Week	Control 1 (saline) (*n*=8)	Control 2 (control virus) (*n*=8)	rAAV2/1-Acrp30-administered (*n*=9)	Control 1 (saline) (*n*=8)	Control 2 (control virus) (*n*=8)	rAAV2/1-Acrp30-administered (*n*=9)
Urine volume (ml)				*Body weight (g)*		
0	67.63 (12.32)	64.39 (7.95)	62.41 (11.89)	371.53 (54.34)	373.90 (71.97)	376.71 (20.42)
4	65.55 (21.64)	60.41 (14.73)	44.80 (9.67) [1]	375.15 (50.34)	377.89 (67.91)	391.63 (13.76)[3]
8	69.41 (15.62)	66.04 (13.71)	27.40 (13.83)[1,2]	380.83 (56.71)	381.83 (59.64)	409.68 (34.20)[4]
Fasting blood glucose (mmol/L)				*2 hour postprandial blood glucose (mmol/L)*		
0	14.13 (5.41)	14.94 (4.93)	13.51 (4.29)	20.03 (5.34)	21.01 (7.32)	19.68 (2.42)
4	13.95 (4.54)	14.16 (6.79)	9.14 (1.57)[1]	18.12 (6.34)	20.62 (8.09)	13.57 (3.61)[3]
8	14.89 (2.38)	14.06 (2.40)	6.51 (0.95)[1,2]	20.25 (5.07)	19.77 (4.45)	9.25 (1.23)[4]
HbA1c (%)				*Serum insulin (uIU/mL)*		
0	8.73 (1.54)	9.03 (2.75)	8.86 (1.61)	59.88 (12.64)	57.42 (10.81)	61.68 (13.42)
4	8.58 (1.39)	8.79 (2.17)	6.94 (1.93)[1]	62.53 (12.37)	59.45 (9.89)	62.57 (14.62)
8	8.93 (1.85)	8.82 (2.03)	4.75 (1.23)[1,2]	65.11 (13.76)	61.83 (10.04)	60.96 (9.05)
Triglycerides (mmol/L)				*Total cholesterol (mmol/L)*		
0	2.71 (0.74)	2.68 (0.81)	2.69 (0.70)	4.46 (1.24)	4.51 (1.07)	4.48 (1.15)
4	2.68 (0.33)	2.71 (0.45)	1.85 (0.76)[1]	4.67 (0.28)	4.53 (0.13)	3.32 (0.97)[1]
8	2.73 (0.81)	2.72 (0.74)	0.65 (0.12) [1,2]	4.95 (1.04)	4.89 (1.12)	2.58 (1.16)[1,2]
Low-density lipoprotein (mmol/L)				*High-density lipoprotein (mmol/L)*		
0	1.54 (0.63)	1.57 (0.56)	1.56 (0.49)	0.49 (0.18)	0.42 (0.12)	0.47 (0.14)
4	1.59 (0.46)	1.58 (0.32)	1.16 (0.41)[1]	0.44 (0.03)	0.45 (0.08)	1.19 (0.12)[3]
8	1.53 (0.82)	1.60 (0.82)	0.62 (0.05)[1,2]	0.81 (0.26)	0.84 (0.10)	1.28 (0.71)[3]

[1]p<0.05 A group (4 weeks, 8 weeks) *v.* C1, C2 group (4 weeks, 8 weeks); [2]p<0.05 A group (4 weeks, 8 weeks) *v.* A group (0 week, 4 weeks) ; [3]p<0.05 A group (4 weeks) *v.* C1, C2 group (0 week); [4]p<0.05 *v.* A group (8 weeks) *v.* C1, C2 group (8 weeks)

Effect of rAAV2/1-Acrp30 on the ultrastructure of the aorta

The results of light microscopy showed that endothelial cells in the aortic tunica intima and smooth muscle cells with eosinophilic cytoplasm in the tunica media decreased. These groups also had a number of foam cells full of fatty vacuoles (Fig. 2A-D). In the rAAV2/1-Acrp30-administered group, the endothelial cells of the aorta were almost integrated and proliferated with eosinophilic cytoplasm and monolayer endothelial cells closely stuck to the integrated internal elastic lamina. Endothelial cells and smooth muscle cells in the tunica media were arranged in an integrated manner almost without fatty vacuoles and foam cells (Fig. 2E-F).

Electron microscopy showed that the tunica intima in the aorta in the control groups showed significant pathological changes, whereas some tunica intima were not smooth with much lipid and the internal elastic lamina of the aorta was not integrated. It was accompanied with a lot of fatty vacuoles in the smooth muscle cells of tunica intima and even with formation of foam cells (Fig. 3A-D).

In the rAAV2/1-Acrp30-administered group, the smooth tunica intima in the aorta displayed a restoring state and the endothelial cells increased and were arranged almost regularly with few inflammatory cells and lipidoses. There were various kinds of cell organelles and integrated internal elastic lamina in the cytoplasm of endothelial cells and smooth muscle cells without fatty vacuoles. The arrangement of spindle-shaped endothelial cells was regular without widened intercellular spaces, which suggested that the tunica intima in the aorta was recovering to normal (Fig. 3E-F).

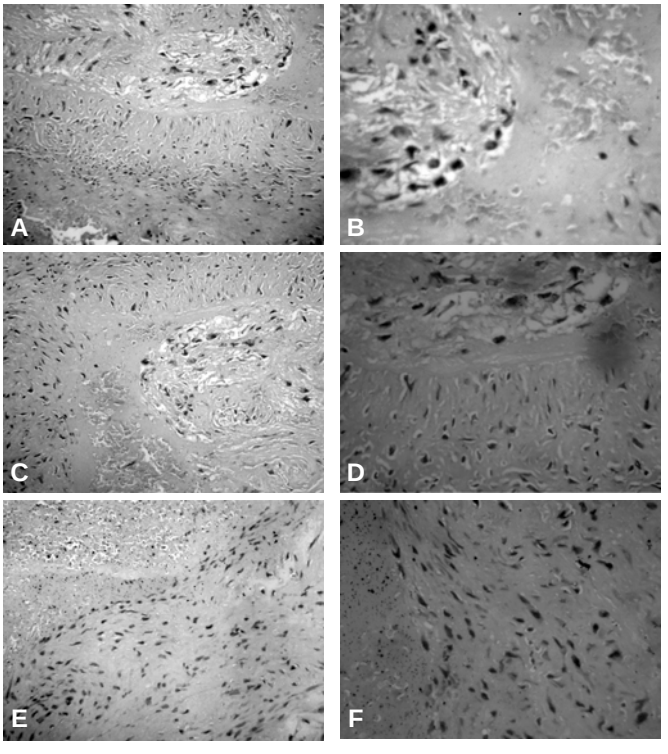

FIG 2. Architecture of rat aorta by light microscope. A. Control group 1 (×200); B. Control group 1 (×400); C. Control group 2 (×200); D. Control group 2 (×400); E. rAAV2/1-Acrp30-administered group (×200); F. rAAV2/1-Acrp30-administered group (×400)

DISCUSSION

Adiponectin is a 244-amino acid polypeptide which is known as adipo Q, AcrP30, apM1 or gelatin binding protein-28 (GBP-28).[8] It is an adipose tissue-specific collagen-like molecule and expressed exclusively in adipocytes which can improve insulin resistance and increase the sensitization of insulin. Adiponectin can also modulate a number of metabolic processes, including glucose regulation and fatty acid catabolism.[9] It also has anti-inflammatory effects on the endothelium in the blood vessels. Low adiponectin levels developed in patients with type 2 diabetes. Some studies[10–12] have reported that low adiponectin was a risk

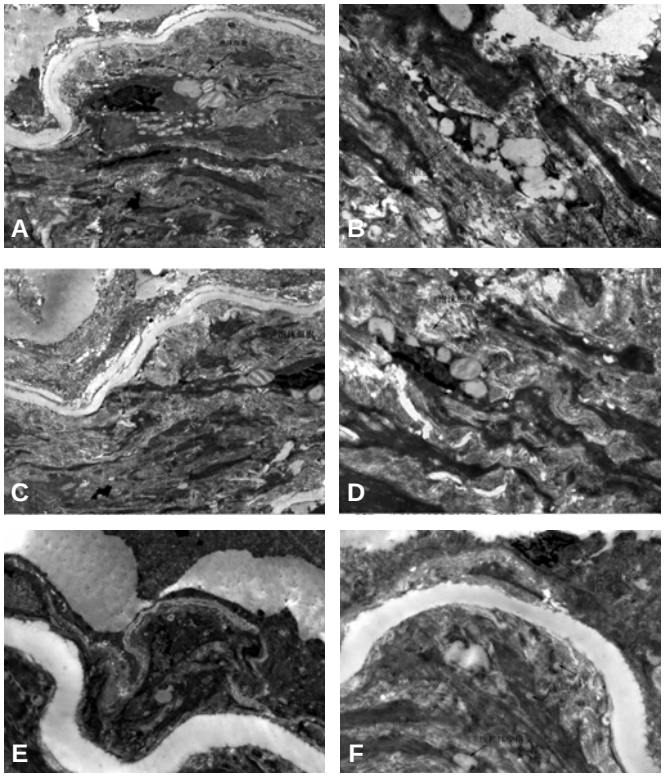

FIG 3. Ultrastructure of the rat aorta by electron microscope.
A. Control group 1 (×5000); B. Control group 1 (×10 000);
C. Control group 2 (×5000); D. Control group 2 (×10 000);
E. rAAV2/1-Acrp30-administered group (×5000);
F. rAAV2/1-Acrp30-administered group (×10 000)

factor for coronary artery disease and cardiovascular events. Patients with type 2 diabetes with macrovascular disease have lower adiponectin than those without macrovascular disease.[13] In this study, we studied effects of the recombinant adeno-associated virus vector of adiponectin (AAV2/1-Acrp30)[5] on glucose and lipid metabolism and the ultrastructure of the aorta of GK rats.

Akin to previous studies,[8,14,15] we found that in the rAAV2/1-Acrp30 administered group, the fasting and postprandial blood sugar levels were lower but the insulin levels were not. This was perhaps related with adiponectin promoted secretion of insulin and improved sensitivity of insulin.[16] Adiponectin (by rAAv2/1-Acrp30) could decrease the expression of phosphoenolpyruvate and sterol regulatory element-binding protein-1C in the liver, leading to an increase in the uptake, disposal and utilization of glucose in peripheral tissues. Hence, we surmised that adiponectin improved blood sugar by the therapeutic effect of rAAV2/1-Acrp30 on the liver and muscular tissues and the recovery of sensitivity of insulin in liver and muscular tissues.

We observed that adiponectin reduced the deposition of lipid droplets in cells by regulating the lipid metabolism—decreasing TC, TG and LDL, and increasing HDL. Anti-lipid peroxidation could mitigate the damage of the endothelial cells and inhibit the hyperplasia of vascular smooth muscle to interrupt arteriosclerosis. Luo *et al.*[16] showed that adiponectin could protect macrophage transformation into foam cells by inhibiting the aggregation of lipid and expression of scavenger receptor A on the macrophage. In our study, the ultrastructure of aorta in the rAAV2/1-Acrp30 administered group was restored towards normal. The mechanism was possibly related to the effect of adiponectin on glucose and lipid metabolism, and especially to the gene targeting therapy by rAAV2/1-Acrp30. This possibly resulted in adiponectin and adiponectin receptors being linked to endothelial cells in the aorta by a special saturated means to suppress the formation of foam cells and protect the aorta. There are two kinds of receptors of adiponectin which can express in atherosclerotic plaques and macrophages.[17]

The exact mechanism of adiponectin on diabetic macroangiopathy remains unknown. Endothelial dysfunction was considered to be an early event and played a critical role in the development of atherosclerosis. Cao *et al.*[18] observed that treatment of adiponectin knockout [adipo(–/–)] mice with adiponectin reduced the production of superoxide in the aorta, increased the phosphorylation of eNOS, normalized vasodilatation responses to acetylcholine and thereby may improve endothelial function. Adiponectin strongly inhibits expression of adhesion molecules including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and E-selectin.[19] It also inhibits the activation of nuclear factor kappa B by inhibiting TNF-alpha,[20] which may in turn inhibit monocytes from adhering to endothelial cells and thus prevent the change in endothelial function that results in atherosclerosis. Adiponectin inhibits the synthesis and release of IL-8, a pro-inflammatory chemokine with a role in atherogenesis, by inhibiting a PKA-dependent NF-kappa B signalling pathway, and thus might prevent atherosclerosis.[21] Adiponectin also suppresses the uptake of oxidized LDL and the transformation from macrophages to foam cells resulting in atherosclerosis by inhibiting expression of Scavenger receptor class A1 macrophages (SR-A).[22] Thus, adiponectin may protect injured arterial walls against atherosclerosis. Our study suggests that gene therapy by adiponectin-containing rAAV2/1-Acrp30 may be associated with improvement in blood glucose and lipid parameters and reduction in the deposition of lipid droplets in aorta and thus protect endothelial function and alleviate atherosclerosis of aorta.

Conflict of interest. None.

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