

Originals

Effect of Poly (ADP-ribose) Polymerase Inhibitors on Hypoadiponectinemia Caused by Chronic Blockade of Nitric Oxide Synthesis in Rats

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SUMMARY

Oxidant stress-induced activation of poly (ADP-ribose) polymerase (PARP) contributes to the pathogenesis of various cardiovascular diseases. Adiponectin is an adipocyte-derived anti-atherogenic protein. In the present study, we investigated the role of PARP in the development of hypoadiponectinemia caused by chronic blockade of nitric oxide synthesis with N^o-nitro-L-arginine methyl ester (L-NAME) in rats. Decreased production of NO and increased production of O₂⁻ were observed in aortas from L-NAME-treated rats. Plasma adiponectin levels and adiponectin mRNA levels within adipose tissue were markedly decreased in L-NAME-treated rats. Concurrent administration of potent PARP inhibitors, INO-1001 or PJ34, with L-NAME did not restore plasma adiponectin levels or fat adiponectin mRNA levels. Thus, the cardiovascular protective effects of PARP inhibitors are not associated with adiponectin levels. Therefore, drugs which increase adiponectin levels may be beneficial alongside PARP inhibitors in the treatment of cardiovascular disease.

Key Words : adiponectin, poly (ADP-ribose) polymerase, oxidative stress, adipocytokine

INTRODUCTION

Activation of poly (ADP-ribose) polymerase (PARP) is now considered a final common effector in various types of tissue injury, including systemic inflammation, circulatory shock and ischemia/reperfusion¹⁾. Free radical and oxidant production and related cytotoxicity during ischemia/reperfusion leads to DNA strand breakage, which activates the nuclear enzyme PARP and initiates an energy-consuming, inefficient cellular metabolic cycle, with transfer of the ADP-ribosyl moiety of NAD⁺ to protein acceptors¹⁾. A growing number of studies of cell cul-

tures and rodents, as well as pre-clinical studies of large animals, demonstrate beneficial effects of PARP inhibitors in limiting regional and global ischemia/reperfusion injury^{2,3)}.

Adiponectin is abundantly expressed in adipose tissue. Decreased adiponectin levels are observed in states of increased insulin resistance, such as obesity, type 2 diabetes, coronary artery disease and hypertension^{4~6)}. In addition to metabolic effects, adiponectin protects vessels from damage through various mechanisms. Recently, we reported that angiotensin II-induced oxidative stress elicits hypoadiponectinemia in rats⁷⁾. In addition, hypoadiponectinemia in obesity is caused by increased oxidative stress in accumulated fat⁸⁾.

In the present study, potent PARP inhibitors, INO-1001 and PJ34^{2,3)}, were used to investigate the role of PARP in the development of hypoadiponectinemia caused by

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chronic blockade of nitric oxide synthesis with N^o-nitro-L-arginine methyl ester (L-NAME) in rats. L-NAME both inhibits NO production and causes oxidative stress possibly producing hypoadiponectinemia in rats.

MATERIALS AND METHODS

Animal model of inhibition of NO synthesis. The present experiments were reviewed and approved by the Committee on Ethics of Animal Experiments, and were conducted according to the Guidelines for Animal Experiments, at Dokkyo University Faculty of Medicine.

Fourteen week-old male Wistar-Kyoto rats were housed singly in a pyrogen-free facility. Four groups of rats were studied. The first (control) group received untreated laboratory chow and drinking water. The second group (L) received L-NAME in the drinking water (1 mg/mL). At this concentration, the daily intake of L-NAME was 100 mg/kg per day^{9,10}. The third group (INO/L) received INO (0.3 mg/ml) and L-NAME (1 mg/ml), and the fourth group (PJ/L) received PJ (0.2 mg/ml) and L-NAME (1 mg/ml), in their drinking water.

Vessel Harvesting and preparation. On day 5 of treatment, we measured heart rate and systolic blood pressure by the tail-cuff method. The rats were anesthetized with intraperitoneally administered pentobarbital, after which their chests were opened. With the heart still beating, heparin (150 IU) was given via intracardiac injection. The thoracic aortas were removed en bloc and placed in cold Krebs-Henseleit solution. Extravascular tissue was rapidly removed, after which the vessel lumens were flushed with solution. In some rats, the aortas were cut into three 5-mm ring segments for the use in studies of NO production, as well as superoxide anion production.

Measurement of vascular superoxide anion and NO production from the aorta. Superoxide anion production was measured using lucigenin chemiluminescence, as previously described¹¹. Briefly, the thoracic aortas were carefully dissected and cleared of perivascular tissue and blood contaminants under a microscope, after which they were placed in HEPES-buffered physiological salt solution (in mmol/L: NaCl 121, KCl 4.7, NaHCO₃ 24.7, MgSO₄ 12.2, CaCl₂ 2.5, KH₂PO₄ 1.2, and glucose 5.8, aerated with 95% O₂ and 5% CO₂). In a preliminary study,

we confirmed no adhesion of inflammatory cells to the endothelium (data not shown). Scintillation vials containing 1 mL HEPES-buffered PSS with 5 μmol/L lucigenin (bis-N-methylacridinium nitrate) were placed into a scintillation counter (Luminescence Reader BLR 301, ALOKA). We used Tiron (10 mmol/L), a superoxide scavenger, in all experiments to confirm the validity of our technique with lucigenin. After dark adaptation, background counts were recorded for three minutes and then three vascular segments (5 mm in length) from each thoracic aorta were added to the vial. Scintillation counts were then recorded every minute for 10 minutes and the respective background counts were subtracted. The vessels were then dried for determination of dry weight. Lucigenin counts were expressed as counts per minute per milligram of dry weight.

A chemiluminescence-based NO analyzer (270B, Sievers) was used to measure NO production, as previously described¹². Briefly, the 5-mm ring segments of the aorta were incubated in 2 mL of Hanks' balanced salt solution containing a calcium ionophore A23187 (1 μmol/L) and L-arginine (100 μmol/L). Specific NO-generating capacity was expressed as nanomoles per hour per dry weight.

Measurement of plasma NO. Plasma nitrite and nitrate levels (NO₂⁻ and NO₃⁻) were measured with an automated NO detector/high-performance liquid chromatography system (ENO10, Eicom Co).

Measurement of plasma adiponectin levels. Plasma adiponectin levels were determined by ELISA using a kit for measurement of rat/mouse adiponectin (Otsuka pharmaceuticals).

Measurement of adiponectin mRNA levels in adipose tissue. Standard Northern blotting was used to investigate the mRNA expression of adiponectin in adipose tissues. After probing for adiponectin, filters were stripped and re-probed for the presence of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

Quantification of adiponectin mRNA was performed by amplification of cDNA using a LineGene real-time thermocycler (BioFlux). Message copy numbers were obtained from standard curves generated from genuine rat adiponectin. Similarly, PARP mRNA levels were mea-

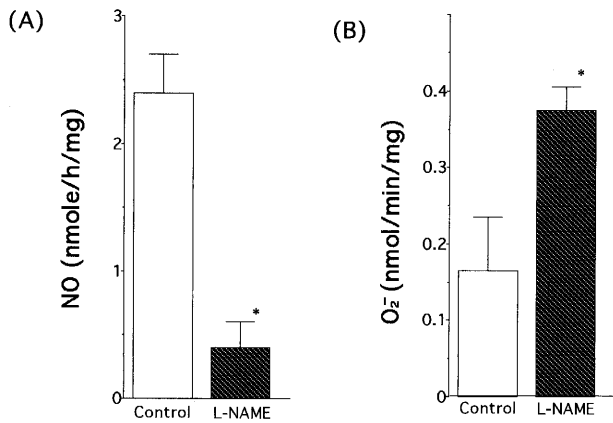


Fig. 1 NO (A) (nanomoles per hour per milligram tissue) and O₂⁻ (B) (nanomoles per 10 minutes per milligram tissue) production in rat aortic segments. **P* < 0.01 vs control group. Each bar represents *n* = 6.

sured in adipose tissue. Adiponectin mRNA levels were expressed as a ratio of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA level. The following primer sequences were used for real-time PCR: Adiponectin, 5'-AGAGATGGCACTCCTG-3' and 5'-AGATCTTAGTAAAGCGAATG-3'; PARP, 5'-CTGGAGAAAGCGGGAAGGT-3' and 5'-TTGGACGGCATCTGCTCAAG-3'; and GAPDH, 5'-AGTTCAAGAACGCCTTACCA-3' and 5'-GCTCGCACACGGAAGTT-3'.

Statistical Analysis. Data are expressed as mean values ± SEM. Differences between two experiments were compared by Student's *t* tests. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Body weight and hemodynamic parameters

During the 5-day treatment period, body weights did not differ significantly between the control and L groups. Significant differences in body weights were also not observed between the INO and INO/L groups, or the PJ and PJ/L groups, which did not differ significantly from the control group. During this period, however, the L group exhibited a significant rise in systolic arterial pressure, compared with the control group. Significantly greater systolic blood pressures were also observed in the INO/L, PJ, and PJ/L groups, compared to controls.

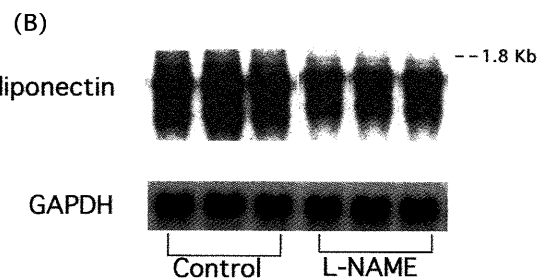
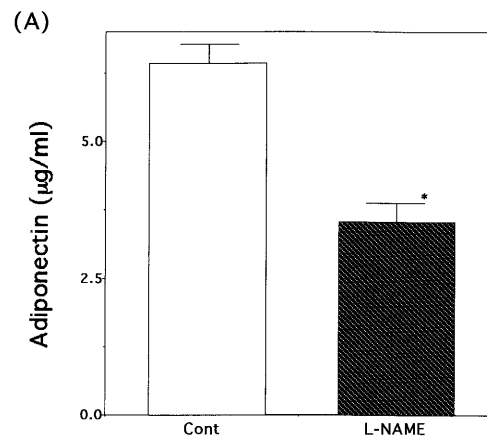


Fig. 2 Plasma adiponectin concentration (*n* = 8) (A) and adiponectin mRNA expression in adipose tissue by northern blot analysis (*n* = 3) (B). **P* < 0.01 vs control group.

Aortic NO and O₂⁻ production and plasma NO_x concentration

NO production was markedly decreased in the L group, compared to the control group (Fig. 1 A). Production of O₂⁻ was greater in the aortic segments of the L group, compared to the control group (Fig. 1 B).

Plasma adiponectin and adiponectin mRNA levels in adipose tissue

Plasma adiponectin was significantly lower in the L group at $3.20 \pm 0.83 \mu\text{g/ml}$, compared to $6.48 \pm 0.33 \mu\text{g/ml}$ in the control group (*p* < 0.005) (Fig. 2 A).

A high level of adiponectin mRNA was observed in the abdominal adipose tissue of control rats, while mRNA expression was clearly reduced in the L group (Fig. 2 B).

No differences in PARP mRNA expression were observed in the adipose tissue of control and L group rats (data not shown).

Effect of INO and PJ on plasma adiponectin concentrations and adiponectin mRNA levels in adipose tissue

To examine the effects of INO and PJ on plasma adiponectin levels and adiponectin mRNA levels in adi-

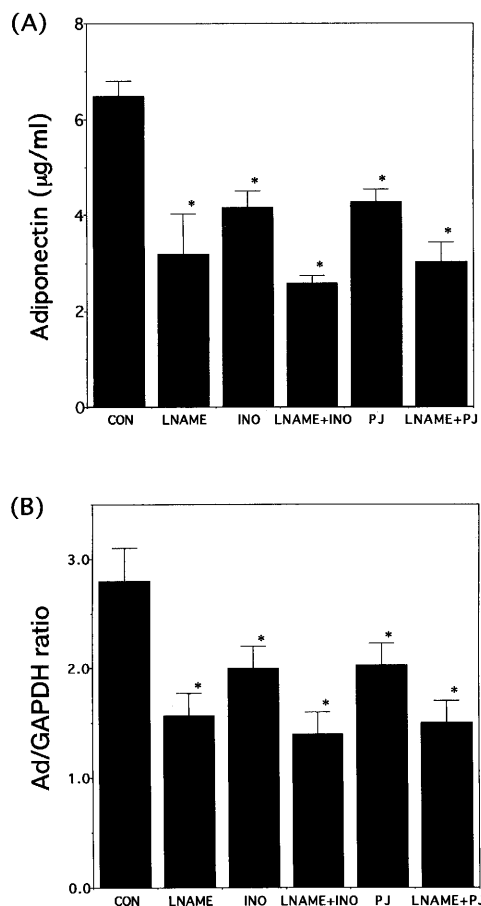


Fig. 3 Effects of INO and PJ on plasma adiponectin concentration ($n = 8$) (A) and adiponectin (Ad) mRNA levels in adipose tissue ($n = 3$) (B). * $P < 0.01$ vs control group.

pose tissue, INO and PJ were administered to rats with or without L-NAME for 5 days. INO or PJ alone decreased rat adiponectin levels and further reduced them to a greater extent than that observed in rats treated with L-NAME alone (Fig. 3 A). Similarly, INO or PJ alone decreased adiponectin mRNA levels in adipose tissue, and further reduced them to a greater extent than observed in rats treated with L-NAME alone (Fig. 3 B).

DISCUSSION

The present study was performed to examine plasma adiponectin levels in rats with blockade of NO synthesis following administration of an inhibitor of NO synthesis, L-NAME. We found decreased plasma levels of adiponectin in rats treated with L-NAME. We also observed decreased levels of adiponectin mRNA in the adipose tissue of rats treated with L-NAME. This decrease in adiponectin is likely the result of oxidative

stress caused by L-NAME. Increased superoxide production was evident within the aorta, and is also likely to occur in the adipose tissue of rats treated with L-NAME. This might be due to decreased adiponectin production since oxidative stress in adipose tissue is known to cause hypoadiponectinemia^{7,8}.

PARP inhibitors ameliorate the development of endothelial dysfunction in rats with endotoxic shock and in mice with diabetes^{13,14}. We examined whether PARP inhibitors might reverse the effects of L-NAME on adiponectin levels in rats. PARP inhibitors alone did not increase, but rather decreased, adiponectin levels. Furthermore, PARP inhibitors did not reverse the development of decreased adiponectin levels in L-NAME-treated rats, but rather, additively decreased adiponectin levels in rats treated with L-NAME. Similarly, PARP inhibitors did not reverse the observed decline in adiponectin mRNA within the adipose tissue of rats treated with L-NAME.

We expected that PARP inhibitors might reverse the development of hypoadiponectinemia in response to L-NAME. However, PARP inhibitors did not reverse hypoadiponectinemia in L-NAME-treated rats and rats even treated with PARP inhibitors alone had decreased adiponectin levels. One explanation for this is that limited PARP expression in adipose tissue. In fact, very low levels of PARP mRNA levels were observed in the adipose tissue and induction of PARP mRNA in response to L-NAME was not observed. PARP is an energy-consuming enzyme, which transfers ADP ribose units to nuclear proteins. As a result, there is a marked decrease in intracellular nicotinamide dinucleotide (oxidized) (NAD⁺) and adenosine 5'-triphosphate (ATP) levels, resulting in cell dysfunction and cell death via necrosis¹. In large adipocytes, troglitazone causes cell death by apoptosis¹⁵. PARP inhibitors do not reverse hypoadiponectinemia, rather, they reduce adiponectin levels by an unknown mechanism. Thus, the cardiovascular protective effects of PARP inhibitors are not due to effects on adiponectin levels. Therefore, drugs which increase adiponectin levels may be beneficial alongside PARP inhibitors in the treatment of cardiovascular disease.

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