	11/28/19	4 Color Fig(s): F1-4	15:35	Art: NR-D-19-01101

Selective accumulation of adiponectin in the cerebral cortex under chronic cerebral hypoperfusion in the rat

Yu Takahashi[a,b], Hideaki Wakita[a], Kenmei Mizutani[c], AQ1 Atsushi Watanabe[d], Shigeru Sonoda[e] and Hidekazu Tomimoto[b]

Adiponectin is a plasma protein predominantly derived from adipocytes. Adiponectin has beneficial properties against diabetes, cardiovascular diseases, and cancer. In experimental acute cerebral ischemia, adiponectin accumulates on vessels in ischemic lesions and has anti-inflammatory protective effects. Chronic cerebral hypoperfusion is associated with white matter lesions and risk of dementia. Chronic cerebral hypoperfusion induced by permanent occlusion of the bilateral common carotid artery can experimentally produce cerebrovascular white matter lesions in the rat brain. Microglia are activated shortly after ischemia and correlate with the severity of white matter and hippocampal tissue damage. These data suggest that the inflammatory response selectively increases white matter and hippocampal damage during chronic cerebral hypoperfusion. However, factors protecting the cerebral cortex have not been elucidated. To clarify the role of adiponectin, we investigated possible changes in adiponectin and adiponectin receptor 1 (ADR1) in the brains of rats under chronic cerebral hypoperfusion. Adiponectin accumulated on the vessels predominantly in the cerebral cortex under chronic cerebral hypoperfusion. Adiponectin accumulation was not detected in the white matter or hippocampus. In the cerebral cortex, the number of ADR1-immunopositive vessels was increased, and adiponectin was colocalized with ADR1. It is plausible that accumulation of adiponectin may be mediated by the binding of adiponectin to ADR1, and its accumulation in the cerebral cortex may protect tissue injury by inhibiting inflammation under chronic cerebral hypoperfusion.

NeuroReport 2019, **XXX**:000–000

Keywords: adiponectin, adiponectin receptor 1, chronic cerebral hypoperfusion, cerebral cortex, hippocampus, white matter

[a]Department of Internal Medicine, Nanakuri Memorial Hospital, Fujita Health University; [b]Department of Neurology, Graduate School of Medicine, Mie University, Mie; [c]Joint Research Support Promotion Facility, Center for Research Promotion and Support, Fujita Health University; [d]Laboratory of Research Advancement, Research Institute, National Center for Geriatrics and Gerontology, Aichi and [e]Department of Rehabilitation Medicine II, School of Medicine, Fujita Health University, Mie, Japan

Correspondence to Hideaki Wakita, Department of Internal Medicine, Nanakuri Memorial Hospital, Fujita Health University, Ohdoricho, Tsu, Mie, Japan
Tel: +81 59 252 1555; fax: +81 59 252 1383; e-mail: wakita@fujita-hu.ac.jp

Received 14 October 2019 **Accepted** 14 November 2019

Introduction

Adiponectin is a plasma protein mainly derived from adipocytes. Adiponectin contributes to the homeostatic control of glucose levels and lipids, energy metabolism, and has anti-inflammatory actions [1,2]. Adiponectin has beneficial properties against diabetes, cardiovascular diseases, and cancer [2]. Adiponectin multimerizes to form stable trimer, hexamer, or high molecular weight complexes in the blood [3]. In the central nervous system (CNS), tightly regulated membrane permeability of cerebral blood vessels permits selective passage of adiponectin trimers and hexamers into the CNS [4]. Thus, the physiological actions of adiponectin are attributed to its trimeric and hexameric forms [5]. Adiponectin is not produced in the brain and is delivered into the brain by the blood stream [4]. Lower plasma adiponectin levels are associated with ischemic cerebrovascular disease [6]. Hypoadiponectinemia has been related to an increased risk of mortality after stroke [7]. The inverse association between plasma adiponectin levels and cerebrovascular disease is in accordance with amelioration of acute ischemic injury by adiponectin, which can be delivered intravenously and suppresses intracerebral inflammatory reactions [8]. Moreover, circulating adiponectin accumulates on blood vessels in ischemic lesions, possibly due to its role in adhering to vascular endothelial cells [9].

There have been conflicting data on the level of adiponectin in patients with cognitive decline and incident dementia, ranging from decreased [10] to increased levels [11]. These inconsistent results may be due to the different stages or underlying causes of dementia. To ascertain the role of adiponectin in dementia of vascular causes, we used a chronic cerebral hypoperfusion model, which induces cognitive decline in the rat by permanent occlusion of both common carotid arteries [12–14]. Prolonged decrease of cerebral blood flow results in the demyelination and axonal loss in white matter, and memory impairment, which is associated with injury to frontal-subcortical circuits [12–14]. The neuronal loss in CA1 hippocampal region has also been reported in this model [14,15]. Although white matter and hippocampus bear most of the obvious histopathological changes after

DOI: 10.1097/WNR.0000000000001391

<zdoi;10.1097/WNR.0000000000001391>

occlusion, it is worth noting that bilateral carotid ligation exposes the entire forebrain to prolonged hypoperfusion.

To clarify the role of adiponectin, we investigated the changes in adiponectin and adiponectin receptor 1 (ADR1) expression in the rat brain under chronic cerebral hypoperfusion. We found increase of adiponectin in vascular endothelial cells. Adiponectin-accumulating vessels were preferentially distributed in the cerebral cortex, while the white matter and hippocampus were spared. These findings reveal regional vulnerability to chronic cerebral hypoperfusion.

Materials and methods

Surgical procedure

The present study used 37 male Wistar rats weighing 250–350 g (Charles River Laboratories Japan, Inc., Japan). The rats were anesthetized with 5% isoflurane for induction and 1.5% isoflurane for maintenance in 30% O_2/70% N_2O through a facemask. During the operation, the core body temperature was maintained at 37.0 ± 0.5°C using a heating pad. Both common carotid arteries were double-ligated with 5-0 silk sutures, as described elsewhere [12]. After the operation, animals were housed in cages with food and water available *ad libitum*. As controls, 8 sham-operated animals (four animals for western blot and four animals for immunohistochemistry) were subjected to the same surgery except for carotid ligation.

The Fujita Health University Institutional Animal Care and Use Committee approved all experiments. All experiments were conducted in accordance with the guidelines for the management of laboratory animals of Fujita Health University.

Western blot

The differences in adiponectin protein levels in the cerebral hemisphere were analyzed 7 days after bilateral carotid occlusion and sham-operation by western blot analysis. Equal amounts of brain proteins were resolved on polyacrylamide gels, and were transferred to a nitrocellulose membrane. The membrane was incubated with the following primary antibodies (dilutions in parentheses): mouse anti-adiponectin antibody (1:500; R & D Systems, Inc., Minneapolis, Minnesota, USA) and mouse anti-actin antibody (1: 2000; IMGENEX Corporation, San Diego, California, USA). Then, the blot was incubated with alkaline phosphatase-conjugated anti-mouse antibodies (1:2000; Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania, USA). An alkaline phosphatase substrate kit (BCIP/NBT; Vector Laboratories, Burlingame, California, USA) was used for the detection of immunoreactive proteins. Comparative quantification was analyzed using Scion Image software (Meyer Instruments Inc., Houston, Texas, USA). These procedures are described in detail elsewhere [16].

Immunohistochemistry

At 1, 3, 7, 14, and 30 days after surgery, the animals were deeply anesthetized with sodium pentobarbital (100 mg/kg, intraperitoneally), perfused transcardially with 0.01 mol/L PBS, and then with a fixative containing 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB).

The coronal blocks were postfixed for 12 h in 4% paraformaldehyde in 0.1 mol/L PB and stored in 20% sucrose in 0.1 mol/L PB for further immunohistochemical analysis. Endogenous peroxidases were inactivated by 30 min incubation in 0.3% hydrogen peroxide in 10% methanol/0.1 mol/L PBS. Then, the sections were incubated for 1 h in 5% normal horse serum in 0.1 mol/L PBS/0.3% Triton X-100 (PBS-Triton) to block nonspecific staining. After blocking, the sections were incubated at 4°C overnight in PBS-Triton containing mouse anti-adiponectin antibody (1:2000) or goat anti-ADR1 antibody (1:5000; abcam, Cambridge, UK). The sections were subsequently incubated for 1 h in PBS-Triton containing biotinylated anti-mouse IgG or biotinylated anti-goat IgG (7.5 mg/ml; Vector Laboratories), followed by incubation with an avidin-biotin-peroxidase complex solution (1:100; Vector Laboratories) for 1 h at room temperature. After each incubation, except for the blocking of nonspecific staining, the sections were washed with PBS-Triton for 15 mins. The immunoreative products were detected with diaminobenzidine (DAB kit; Vector Laboratories).

To confirm the localization of adiponectin on vascular endothelial cells, double-labeling fluorescent immunohistochemistry was used. After blocking nonspecific staining, the sections were incubated at 4°C overnight in PBS-Triton containing mouse anti-adiponectin antibody (1:2000) and rabbit anti-von Willebrand factor antibody (1:1000; Sigma, St Louis, Missouri, USA). Then, the sections were incubated for 1 h in PBS-Triton containing Cy3-conjugated anti-mouse IgG and a fluorescein isothiocyanate (FITC) conjugated anti-rabbit IgG (1:100; Jackson Immuno Research Laboratories).

For the assessment of the colocalization of adiponectin with ADR1, double-labeling fluorescent immunohistochemistry as described above was used. The agents were as follows: mouse anti-adiponectin antibody (1:2000), goat anti-ADR1 antibody (1:5000), Cy3-conjugated anti-goat IgG, and a FITC-conjugated anti-mouse IgG (1:100).

We counted the number of adiponectin or ADR1-immunopositive vessels in the total area of the cerebral cortex, corpus callosum, internal capsule, caudoputamen, optic tract, and hippocampus in the cerebral hemispheres in a stereotactically determined section-2.3 mm from bregma, as described elsewhere [13]. This assessment was performed without information of animal's group allocation.

	11/28/19	4 Color Fig(s): F1-4	15:35	Art: NR-D-19-01101

Statistical analysis

Data are represented as mean ± SD. Differences in adiponectin level between groups were analyzed by unpaired two-tailed Student's *t*-test. Differences in adiponectin or ADR1-immunoreactive vessels were determined by repeated measures analysis of variance (ANOVA) followed by Bonferroni's post-hoc comparisons tests using GraphPad Prism 6 (GraphPad Software, La Jolla, California, USA). $P < 0.05$ was considered statistically significant.

Results

Mortality rate

Of the 37 animals that were subjected to bilateral carotid ligation, five (13.5%) died within 7 days after ligation. None of the sham-operated animals died. Four animals in each group were used.

Effects of chronic cerebral hypoperfusion on adiponectin protein levels in the brain

We assessed the adiponectin levels in the cerebral hemispheres by western blot analysis. Western blot for adiponectin detected two identical bands with molecular weights of 30 kDa and 90 kDa, respectively. These bands were diminished in intensity or undetectable when the antibody was preadsorbed with adiponectin protein (data not shown). The band with a molecular weight of 90 kDa corresponded to the trimer of adiponectin.

To quantify relative levels of adiponectin, we compared band intensities from different groups, normalized to the level of actin as an internal control (Fig. 1). F1

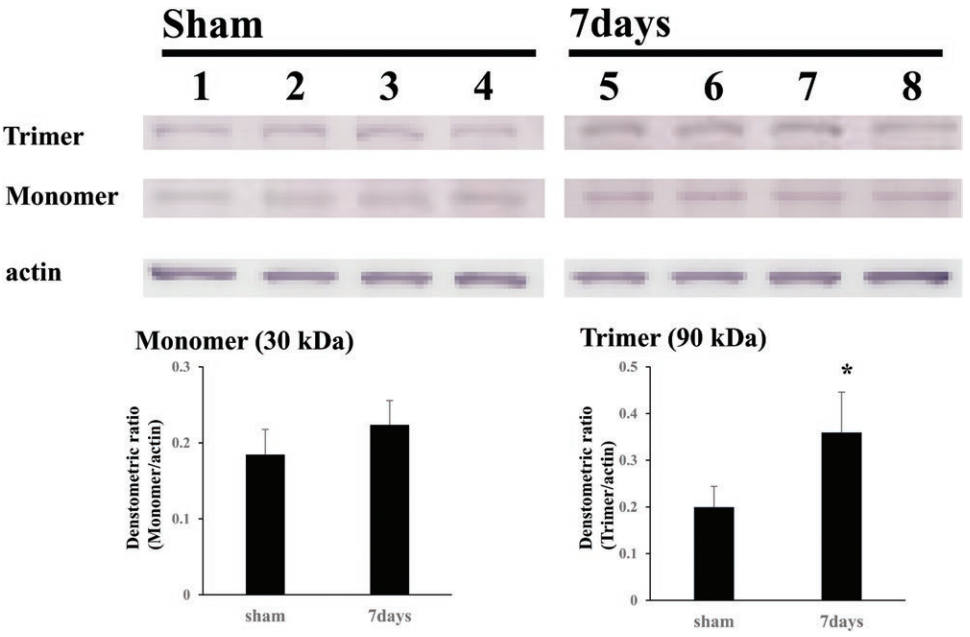
Protein levels of adiponectin trimer in the brains of animals subjected to 7-day bilateral carotid occlusion were significantly increased compared to those of animals subjected to sham-operation ($P < 0.05$).

Spatial and temporal profiles of adiponectin accumulation in the brain

To examine the distribution of adiponectin and ADR1, immunohistochemical analysis was used. Adiponectin-immunoreactive vessels were selectively increased in the cerebral cortex and caudoputamen. In contrast, no increase in adiponectin-immunoreactive vessels was observed in the white matter and hippocampus. The number of adiponectin-immunopositive vessels in the cerebral cortex was significantly increased from 1 to 14 days after surgery compared to sham-operated group ($P < 0.01$). The number of adiponectin-immunopositive vessels in the caudoputamen was significantly increased at 7 days after surgery compared to sham-operated group ($P < 0.05$). Adiponectin-immunopositive vessels were increased predominantly in the cerebral cortex compared to the caudoputamen (Fig. 2). F2

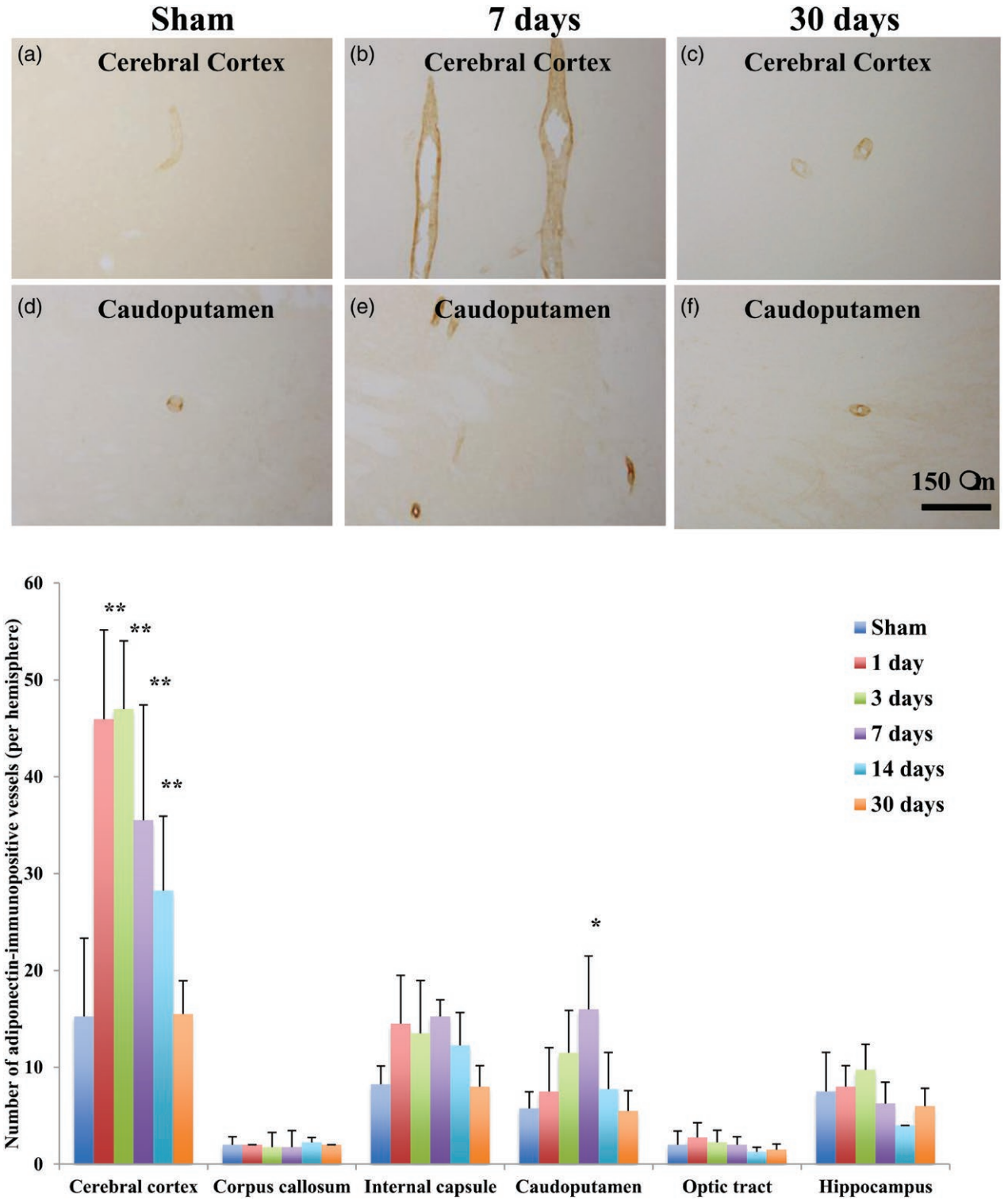
The number of vessels immunopositive for ADR1 in the cerebral cortex was significantly increased from 1 to 14

Fig. 1



Western blot analysis of brain protein extracts. Western blot of adiponectin detected two identical bands with molecular weights of 30 and 90 kDa, respectively. The band at 90 kDa corresponds to the trimer of adiponectin. Protein levels of adiponectin trimer in the brains of animals subjected to 7-day bilateral carotid occlusion showed a significant increase compared to sham-operated group ($P < 0.05$).

Fig. 2



Photomicrographs of immunohistochemistry for adiponectin and histograms of the numerical density of adiponectin-immunoreactive vessels in the brain. The number of adiponectin-immunoreactive vessels in the cerebral cortex was significantly increased from 1 to 14 days after surgery ($P < 0.01$). The number of adiponectin-immunoreactive vessels in the caudoputamen was significantly increased at 7 days after surgery ($P < 0.05$). No increase in adiponectin-immunoreactive vessels was observed in the white matter and hippocampus. *$P < 0.05$ and **$P < 0.01$ compared with sham-operated group. Scale bar, 150 μm.

days after surgery compared to sham-operated group ($P < 0.01$) (Fig. 3). No increase was observed in other regions.

To assess the localization of adiponectin on the vessels, double-label fluorescent immunohistochemistry was performed. Adiponectin accumulated on endothelial cells of the brain vessels (Fig. 4).

To examine the colocalization of adiponectin with ADR1, double-label fluorescent immunohistochemistry was performed. Adiponectin and ADR1 were colocalized on the cerebral vessels under chronic cerebral hypoperfusion (Fig. 4).

Discussion

In this study, we observed that adiponectin trimers selectively accumulated on the vessels in the cerebral cortex and caudoputamen under chronic cerebral hypoperfusion. The increase in adiponectin-immunoreactive vessels in the cerebral cortex persisted from 1 to 14 days after the surgery, whereas the increase in adiponectin-immunoreactive vessels in the caudoputamen was only detected at 7 days after operation, and was not observed in the white matter and hippocampus. Conversely, the number of vessels immunopositive for ADR1 was increased in the cerebral cortex but was not detected in other regions. Double-label immunohistochemistry revealed that adiponectin and ADR1 were colocalized on the cerebral vessels under chronic cerebral hypoperfusion. Therefore, the accumulation of adiponectin seems to be mediated by binding of adiponectin to ADR1, which is expressed on endothelial cells. The number of adiponectin-immunoreactive, but not ADR1-immunoreactive, vessels was significantly increased in the caudoputamen. Accumulation of adiponectin in the caudoputamen may be partly attributable to other receptors, such as ADR2 and T-cadherin.

In this model, protracted hypoperfusion induced delayed white matter lesions [12–14]. In white matter lesions, induction of E-selectin on endothelial cells and infiltration of T cells into the brain parenchyma occurred under chronic hypoperfusion [12,13]. Microglia were activated in a manner that predicted the severity of white matter damage [12]. In addition, inflammatory reactions and neuronal loss were detected in the CA1 subregion of the hippocampus [15]. These data suggest that inflammatory reactions augment white matter and hippocampal damage under chronic cerebral hypoperfusion. Although white matter and hippocampus bear most of the obvious histopathological changes after occlusion, it is worth noting that bilateral carotid ligation exposes the entire forebrain to prolonged hypoperfusion. Therefore, neuroprotective factors may determine regional differences in tissue vulnerability to chronic ischemic insults. Nevertheless, these factors have not been clarified.

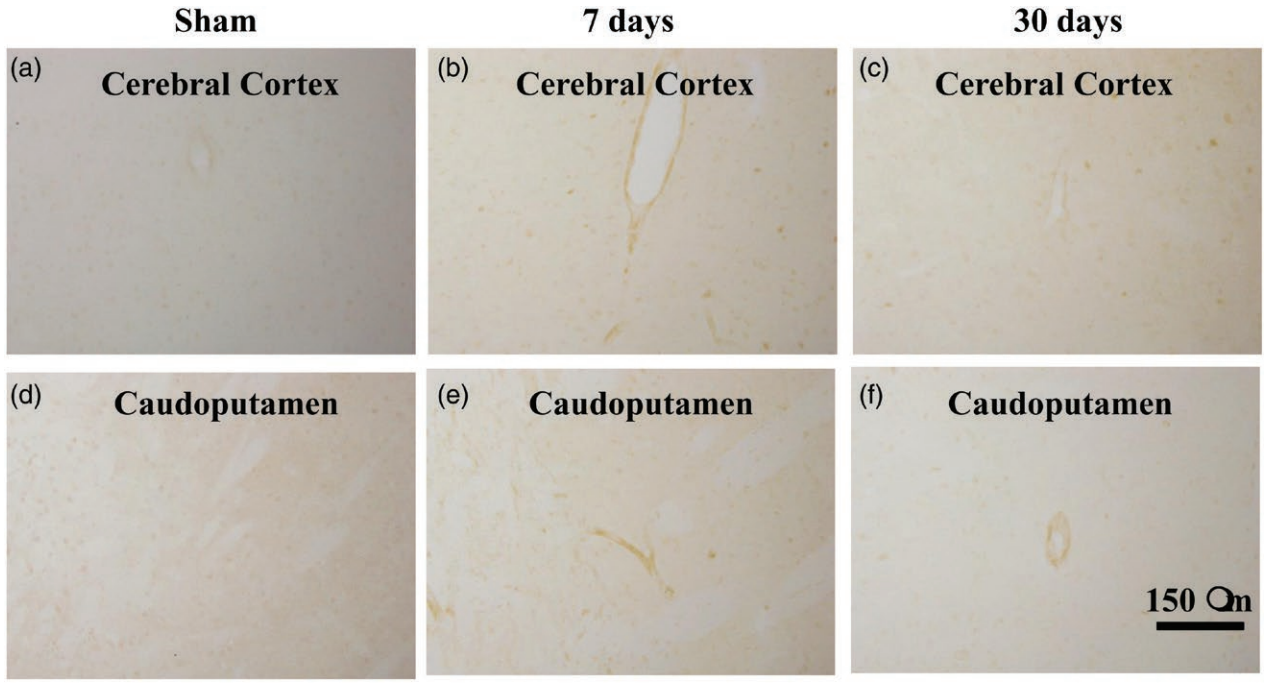
In this study, the number of ADR1-immunopositive vessels was increased in the cerebral cortex under chronic hypoperfusion, and adiponectin was colocalized with ADR1-imunopositive vessels. Adiponectin interaction with ADR1 stimulates AMP-activated protein kinase (AMPK) [4], which subsequently promotes the differentiation of endothelial cell into capillary-like structures [17]. Adiponectin also activates nitric oxide (NO) production in endothelial cells through AMPK-endothelial nitric oxide synthase (eNOS)-dependent signaling pathways [18]. Endothelial NOS exerts vasodilatation and vasculoprotective effects, thereby protecting the brain from cerebral ischemia. Accordingly, compared to wild-type (WT) mice, adiponectin-deficient ($Ad^{-/-}$) mice showed increased brain infarction after ischemia-reperfusion. Phosphorylation of eNOS in ischemic brain tissue and production of NO metabolites in plasma were attenuated in $Ad^{-/-}$ mice compared with WT mice [19]. Adiponectin may protect the cerebral cortex by vasculoprotection through eNOS-dependent mechanisms. In accordance with this hypothesis, regional cerebral blood flow in the cerebral cortex and caudoputamen recovered more rapidly after bilateral carotid occlusion [20]. Alternatively, adiponectin may protect the cerebral cortex by antiapoptotic effects. Administration of recombinant adiponectin attenuated neuronal apoptosis induced by hypoxia-ischemia by activating the AdipoR1/APPL1/LKB1/AMPK signaling pathway in rat pups [21].

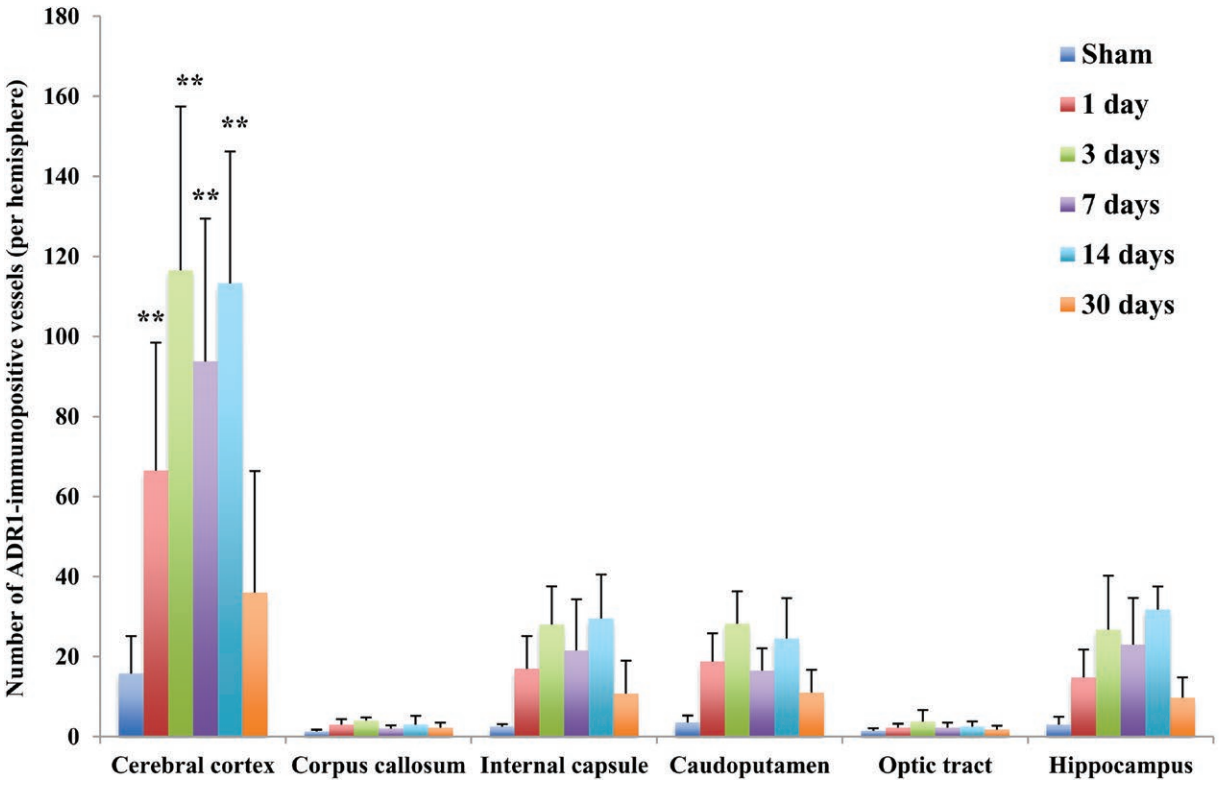
Notably, adiponectin has protective effects against acute ischemic neuronal injury through anti-inflammatory actions [8]. $Ad^{-/-}$ mice exhibit increased leukocyte-endothelium interactions and upregulation of E-selectin and vascular cell adhesion molecule-1 in the vascular endothelium [22]. Indeed, in chronic cerebral hypoperfusion, induction of E-selectin on endothelial cells and infiltration of T cells into the brain parenchyma were less prominent in the cerebral cortex than in the white matter [12,13]. Similarly, an inverse relationship between adiponectin expression and tissue injury has been underscored by the absence of microglial activation and tissue damage in the cerebral cortex [12,14] and less prominent damage in the caudoputamen than in the white matter [12], In addition, inflammatory reactions and CA1 neuronal damage were detected in the hippocampus [15], in which adiponectin was not increased. These data collectively suggest that adiponectin has the potency to protect the cerebral cortex and caudoputamen by suppressing local vessel injuries and surrounding inflammatory processes.

Conclusion

In conclusion, we observed that adiponectin-accumulating vessels in the cerebral cortex were significantly increased from 1 to 14 days after surgery. In contrast, no increase in adiponectin-immunoreactive vessels was observed in the white matter and hippocampus. Inflammatory reactions and tissue damage were less prominent in the cerebral cortex than in the white matter and hippocampus. These results suggest that increase of

Fig. 3

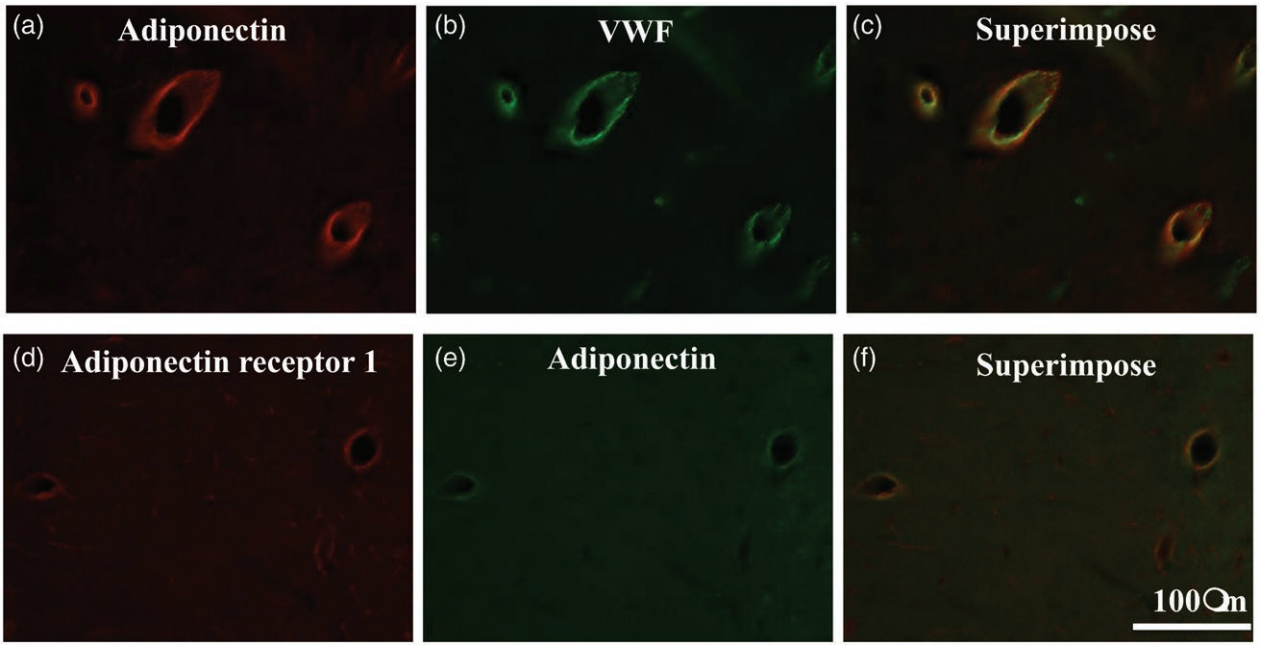




Photomicrographs of immunohistochemical staining for adiponectin receptor 1 (ADR1) and histograms of the numerical density of ADR1-immunoreactive vessels in the brain. The number of ADR1-immunoreactive vessels in the cerebral cortex was significantly increased from 1 to 14 days after surgery. No increase was observed in other regions. $^{**}P < 0.01$ compared to sham-operated group. Scale bar, 150 μm.

	11/28/19	4 Color Fig(s): F1-4	15:35	Art: NR-D-19-01101

Fig. 4



Photomicrographs of double-labeled fluorescent immunohistochemistry of adiponectin with von Willebrand factor and adiponectin with adiponectin receptor 1 (ADR1). Adiponectin and ADR1 were colocalized on the cerebral vessels in chronic cerebral hypoperfusion. Scale bar, 100 μm.

adiponectin in the cerebral cortex may protect against tissue injury by inhibiting inflammatory reactions in chronic cerebral hypoperfusion.

Acknowledgements

The authors are indebted to Mrs. Nagasaka for her excellent assistance.

Conflicts of interest

AQ2 There are no conflicts of interest.

References

1 Goldstein BJ, Scalia R. Adiponectin: a novel adipokine linking adipocytes and vascular function. *J Clin Endocrinol Metab* 2004; **89**:2563–2568.
2 Fang H, Judd RL. Adiponectin regulation and function. *Compr Physiol* 2018; **8**:1031–1063.
3 Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, *et al.* Structure-function studies of the adipocyte-secreted hormone acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. *J Biol Chem* 2003; **278**:9073–9085.
4 Thundyil J, Pavlovski D, Sobey CG, Arumugam TV. Adiponectin receptor signalling in the brain. *Br J Pharmacol* 2012; **165**:313–327.
5 Ebinuma H, Miida T, Yamauchi T, Hada Y, Hara K, Kubota N, Kadowaki T. Improved ELISA for selective measurement of adiponectin multimers and identification of adiponectin in human cerebrospinal fluid. *Clin Chem* 2007; **53**:1541–1544.
6 Chen MP, Tsai JC, Chung FM, Yang SS, Hsing LL, Shin SJ, *et al.* Hypoadiponectinemia is associated with ischemic cerebrovascular disease. *Arterioscler Thromb Vasc Biol* 2005; **25**:821–826.
7 Efstathiou SP, Tsioulos DI, Tsiakou AG, Gratsias YE, Pefanis AV, Mountokalakis TD. Plasma adiponectin levels and five-year survival after first-ever ischemic stroke. *Stroke* 2005; **36**:1915–1919.
8 Chen B, Liao WQ, Xu N, Xu H, Wen JY, Yu CA, *et al.* Adiponectin protects against cerebral ischemia-reperfusion injury through anti-inflammatory action. *Brain Res* 2009; **1273**:129–137.
9 Yatomi K, Miyamoto N, Komine-Kobayashi M, Liu M, Oishi H, Arai H, *et al.* Pathophysiological dual action of adiponectin after transient focal ischemia in mouse brain. *Brain Res* 2009; **1297**:169–176.
10 Teixeira AL, Diniz BS, Campos AC, Miranda AS, Rocha NP, Talib LL, *et al.* Decreased levels of circulating adiponectin in mild cognitive impairment and Alzheimer's disease. *Neuromolecular Med* 2013; **15**:115–121.
11 Une K, Takei YA, Tomita N, Asamura T, Ohrui T, Furukawa K, Arai H. Adiponectin in plasma and cerebrospinal fluid in MCI and Alzheimer's disease. *Eur J Neurol* 2011; **18**:1006–1009.
12 Wakita H, Tomimoto H, Akiguchi I, Kimura J. Glial activation and white matter changes in the rat brain induced by chronic cerebral hypoperfusion: an immunohistochemical study. *Acta Neuropathol* 1994; **87**:484–492.
13 Wakita H, Ruetzler C, Illoh KO, Chen Y, Takanohashi A, Spatz M, Hallenbeck JM. Mucosal tolerization to E-selectin protects against memory dysfunction and white matter damage in a vascular cognitive impairment model. *J Cereb Blood Flow Metab* 2008; **28**:341–353.
14 Farkas E, Luiten PG, Bari F. Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. *Brain Res Rev* 2007; **54**:162–180.
15 Bang J, Jeon WK, Lee IS, Han JS, Kim BY. Biphasic functional regulation in hippocampus of rat with chronic cerebral hypoperfusion induced by permanent occlusion of bilateral common carotid artery. *Plos One* 2013; **8**:e70093.
16 Mizutani K, Sonoda S, Yamada K, Beppu H, Shimpo K. Alteration of protein expression profile following voluntary exercise in the perilesional cortex of rats with focal cerebral infarction. *Brain Res* 2011; **1416**:61–68.
17 Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, *et al.* Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and akt signaling in endothelial cells. *J Biol Chem* 2004; **279**:1304–1309.
18 Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem* 2003; **278**:45021–45026.

19 Nishimura M, Izumiya Y, Higuchi A, Shibata R, Qiu J, Kudo C, *et al*. Adiponectin prevents cerebral ischemic injury through endothelial nitric oxide synthase dependent mechanisms. *Circulation* 2008; **117**: 216–223.
20 Otori T, Katsumata T, Muramatsu H, Kashiwagi F, Katayama Y, Terashi A. Long-term measurement of cerebral blood flow and metabolism in a rat chronic hypoperfusion model. *Clin Exp Pharmacol Physiol* 2003; **30**:266–272.
21 Xu N, Zhang Y, Doycheva DM, Ding Y, Zhang Y, Tang J, *et al*. Adiponectin attenuates neuronal apoptosis induced by hypoxia-ischemia via the activation of adipor1/APPL1/LKB1/AMPK pathway in neonatal rats. *Neuropharmacology* 2018; **133**:415–428.
22 Ouedraogo R, Gong Y, Berzins B, Wu X, Mahadev K, Hough K, *et al*. Adiponectin deficiency increases leukocyte-endothelium interactions via upregulation of endothelial cell adhesion molecules in vivo. *J Clin Invest* 2007; **117**:1718–1726

AUTHOR QUERY FORM

LIPPINCOTT
WILLIAMS AND WILKINS

JOURNAL NAME: WNR

ARTICLE NO: NR-D-19-01101

QUERIES AND / OR REMARKS

QUERY NO.	Details Required	Author's Response
Q1	For indexing purposes, please confirm that author names have been correctly identified as given names (blue) and surnames (red). Color in the byline will not appear on the final published version.	
Q2	Kindly confirm the 'conflicts of interest' statement.	