CASE SERIES

Intra-arterial oxidative stress correlates negatively with cognitive function and positively with postoperative ischemic lesions in carotid artery stenosis stenting

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ABSTRACT

Background and purpose Carotid plaque contains biologically active substances released into the blood during carotid artery stenting (CAS). The main purpose of this prospective study was to analyse sequential changes in oxidative stress during CAS and their relationship to clinical factors.

Methods Twenty-two consecutive CAS procedures were performed between May 2014 and April 2016. Arterial blood was collected four times: (1) after the sheath insertion without edaravone; (2) pre-angioplasty with edaravone from the carotid artery; (3) after post-stenting angioplasty from an occluded carotid artery; and (4) before sheath removal. Derivatives of reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) were measured photometrically. The relationship between d-ROMs or BAP and preoperatively investigated biochemical parameters, cognitive function, and number of diffusion-weighted image (DWI) high spot lesions was analysed using one-way ANOVA and the Tukey–Kramer HSD test.

Results The d-ROM values for CAS were 355±58.8 Carratelli Units at sheath insertion, 315±57.2 after edaravone infusion, 328±56.8 after post-stenting angioplasty, and 315±53.0 just before sheath removal. The d-ROM values were reduced significantly after edaravone infusion (P<0.05). The BAP at sheath insertion was reduced significantly according to age (P<0.05). The d-ROMs at sheath insertion correlated negatively with the dementia scale and positively with the post-CAS DWI high spot (1.00±1.07; P<0.05). Other biochemical parameters did not correlate with the d-ROM values or BAP.

Conclusion Oxidative stress is correlated negatively with cognitive function and positively with postoperative ischemic lesions. Antioxidant potential decreases with age.

INTRODUCTION

Carotid artery stenosis, coronary artery stenosis, and arteriosclerosis obliterans represent systemic atherosclerosis that occur with ageing. In these three diseases, carotid stenosis is relatively easily treated, and the carotid plaque has been analysed from multiple aspects.

Carotid plaque includes multiple bioactive agents including interleukin-1α, interleukin-1β, interleukin 6, matrix metalloproteinase, macrophages, tumour necrosis factor-α, and lipoprotein-associated phospholipid A2. These factors aggravate plaque formation and inflammation and damage the endothelium directly to produce stroke.

Treatment strategies for carotid artery stenosis are medication, carotid endarterectomy (CEA), and carotid artery stenting (CAS). CAS can relieve stenosis, but this method leaves carotid plaque. In particular, balloon angioplasty of carotid plaque causes the release of intra-plaque bioactive agents into the blood. Oxidative stress damages the endothelium, mitochondrial function, and neurons, and also produces inflammation. Additionally, antioxidant potential is important for diminishing these oxidative stresses. The sequential changes in derivatice reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) during CAS have not been clearly analysed. Analysis of these markers can be done within a few minutes during CAS.

The main purpose of this prospective study was to evaluate the sequential changes in oxidative stress and antioxidant potential in the carotid artery and, in particular, to determine whether oxidative stress is increased after plaque compression. We also analysed the correlation between oxidative stress or antioxidant potential and clinical features.

MATERIALS AND METHODS

This prospective study was approved by the Hirosaki University Ethics Committee and written informed consent was obtained from the patients and/or their family. This study was registered as a prospective clinical study under UMIN-CTR R000016770.

From May 2014 through April 2016 we performed 22 consecutive CAS procedures according to the same protocol. All the patients undergoing CAS had ischemic disease and significant carotid artery stenosis. Eighteen men and four women of mean age 70.9±6.85 years were included (table 1). Stenosis of the internal carotid arteries was measured by preoperative digital subtraction angiography according to the North American Symptomatic Carotid Endarterectomy Trial criteria, and the degree of stenosis was 80.4±12.7%. The indications for CAS...
were in accordance with the criteria of the SAPPHIRE trial; patients with symptomatic carotid artery stenosis of at least 50% of the luminal diameter or asymptomatic stenosis of at least 80% were included.\textsuperscript{15} For detection of cognitive function we used the revised Hasegawa Dementia Scale (HDS-R) and the Mini Mental State Examination (MMSE) 5 days after CAS.\textsuperscript{16–19} We selected high-sensitivity C-reactive protein (hs-CRP), total bilirubin, uric acid, total cholesterol, high density lipoprotein, low density lipoprotein, triglycerides, blood sugar, creatinine and estimated glomerular filtration rate for analysis based on previous reports.\textsuperscript{9 20–24}

All the CAS procedures were performed by a single certified experienced operator (NS) according to the standard technique as follows: dual antiplatelets, statins and proton pump inhibitors were routinely applied preoperatively. All the procedures were carried out under local anesthesia. A 9Fr sheath was inserted into the femoral artery and the first arterial blood sampling was performed (figure 1). We kept the activated clotting time to more than 300s whenever possible. Sixty milligrams of edaravone, which is a free radical scavenger, was routinely infused during the loading of the guiding catheter. A MOMA Ultra and a Carotid GuardWire PS (Medtronic, Minneapolis, Minnesota, USA) were used in this series. After finishing the edaravone infusion, a second blood sample was taken via a guiding catheter in the common carotid artery (figure 1). The internal, external, and common carotid arteries were occluded by a balloon from pre-stenting angioplasty to suction out debris. After post-stenting angioplasty, a third blood sample was taken via a suction catheter in the internal carotid artery (figure 1). Before the sheath removal, a fourth arterial blood sample was taken via the sheath in the femoral artery (figure 1). Thus, arterial blood was collected four times: (1) just after sheath insertion without edaravone from the abdominal artery; (2) pre-angioplasty with 60 mg edaravone from the carotid artery; (3) after post-stenting angioplasty from an occluded internal carotid artery; and (4) before sheath removal. The control group comprised two cases of unruptured cerebral aneurysm, one dural arteriovenous shunt, one cerebral arteriovenous malformation, and one meningioma case. \textsuperscript{*}d-ROM values at sheath insertion correlated negatively with HDS-R and positively with post-CAS DWI high spots (P<0.05). \textsuperscript{†}BAP values at sheath insertion correlated negatively with ageing (P<0.05). \textsuperscript{‡}d-ROM values were significantly reduced compared with the value at sheath insertion (P<0.05). BAP, biological antioxidant potential; CAS, carotid artery stenting; DWI, diffusion-weighted imaging; HDS-R, revised Hasegawa Dementia Scale; MMSE, Mini Mental State Examination; MRI, magnetic resonance imaging; NASCET, North American Symptomatic Carotid Endarterectomy Trial; d-ROMs, derivatives of reactive oxygen metabolites.
removal from the abdominal artery. The d-ROMs and BAP were measured by a photometer system (FREE Carpe Diem, Diacron International, Italy). 25–27

The insertion of the sheath into the femoral artery is an invasive maneuver that has the ability to increase d-ROM values due to psychological stress. We included patients without ischemic disease as controls. The control group consisted of five patients who did not have carotid artery disease and who underwent femoral arterial puncture for other non-ischemic indications without edaravone infusion (table 1).

Within 30 hours after CAS we routinely performed MRI of the brain for detection of procedure-related ischemic lesions (diffusion-weighted images; DWI) and other complications as a standard of care examination for all the patients. All the MRI scans were performed on a 3.0 T MRI machine (Signa HDxt 3.0T, General Electric, Fairfield, Connecticut, USA).

Figure 1  Time axis of treatments and evaluations. The zero of the time frame represents sheath insertion for carotid artery stenting. (A) Intraoperative lateral view of the neck. (B) Postoperative diffusion MRI showing high spot lesion. ①, distal protection balloon; ②, occlusion balloon for external carotid artery; ③, occlusion balloon for common carotid artery; ④, intracarotid artery stent; ⑤, catheter tip for suction of debris.
Ischemic stroke

Changes in d-ROMs and BAP during CAS were analysed by a paired t-test. The relationships between the d-ROMs or BAP and the routine preoperative biochemical parameters, cognitive functions, or the number of DWI high spot lesions were analysed by analysis of variance (figure 1). Data are expressed as mean±SD. Statistical differences between the individual groups in neurological functions were analysed using one-way ANOVA (JMP 12; SAS Institute, Cary, North Carolina, USA). Additionally, the Tukey-Kramer HSD test was performed to distinguish significant differences in sequential changes. A P value of <0.05 was considered statistically significant.

RESULTS

The preoperative HDS-R was 25.0±4.93 and the MMSE was 24.6±4.52. The biological parameters are shown in table 1.

The d-ROMs of the CAS patients were 355±58.8 Carratelli Units (CARR U) after sheath insertion without edaravone (table 1, figure 2). The d-ROMs for the non-ischemic disease controls were 374±41.5 CARR U; the d-ROMs for the CAS patients did not increase (table 1). After edaravone infusion, the d-ROMs decreased significantly to 315±57.2 CARR U (figure 3A, P<0.05 compared with the value after sheath insertion without edaravone). After post-stenting angioplasty, d-ROMs increased to 328±56.8 CARR U in the carotid artery. Before sheath removal, the d-ROM values in the abdominal aorta were 315±53.0 CARR U, a significant reduction compared with the value at sheath insertion (P<0.05 vs first blood sample). The BAP did not change significantly during CAS, and the difference in BAP between CAS patients and controls was not significant (table 1, figure 3B). The internal carotid artery occlusion time for the CAS patients was 13.9±2.9 min.

The BAP values at sheath insertion correlated negatively with ageing (P<0.05, figure 4A). The d-ROM values at sheath insertion correlated negatively with the preoperative HDS-R and positively with the number of post-CAS DWI high spots (P<0.05, figure 4B and C). However, the degree of stenosis, hs-CRP, bilirubin, uric acid, lipids, creatinine, and the estimated glomerular filtration rate did not correlate with d-ROM or BAP values. Additionally, the d-ROM and BAP values did not correlate significantly.

DISCUSSION

In this prospective study there were five major findings: (1) oxidative stress and BAP are similar after sheath insertion in patients undergoing CAS and those with non-ischemic disease; (2) BAP after sheath insertion in patients undergoing CAS decreases with age; (3) preoperative oxidative stress is negatively correlated with cognitive function in patients

![Figure 2](http://jnis.bmj.com/)

**Figure 2** Comparison of preoperative values of (A) derivatives of reactive oxygen metabolites (d-ROMs) and (B) biological antioxidant potential (BAP) in patients undergoing carotid stenosis and patients with other non-ischemic diseases. There were no significant changes between the two groups in d-ROMs or BAP.

![Figure 3](http://jnis.bmj.com/)

**Figure 3** Temporal changes in (A) derivatives of reactive oxygen metabolites (d-ROMs) and (B) biological antioxidant potential (BAP) during carotid artery stenting. The d-ROM values reduced significantly after edaravone infusion. This reduction was diminished due to the post-stenting angioplasty. The BAP value did not show any significant changes.
undergoing CAS; (4) edaravone diminishes the additional oxidative stress due to angioplasty immediately during CAS; and (5) high oxidative stress is positively correlated with post-CAS ischemic lesions.

Oxidative stress in CAS and non-ischemic diseases

Cesarone et al reported mean d-ROMs for normal subjects of 312±49 CARR U and for untreated subjects with intermittent claudication of 404±42 CARR U. Our results for the control group are similar to theirs, but the values for our patients treated with CAS are quite different. Our patients undergoing CAS were already being treated with statin, which reduces oxidative stress, so the preoperative oxidative stress for the patients treated with CAS did not increase. This reduction in preoperative oxidative stress in patients undergoing CAS is one of the limitations of this study. The d-ROM values were significant in several ways. Kotani et al reported that intima-media thickness relates significantly to d-ROMs in hypercholesterolemic patients. They also reported a significant positive correlation between d-ROMs and hs-CRP during 1 year in healthy non-mediated subjects. Kamezaki et al reported that d-ROMs correlated significantly with hs-CRP in coronary artery diseases, and increased d-ROMs correlated with a high risk of cardiovascular events. High d-ROM values correlated with an advanced atherosclerotic state and active inflammation. In our study, hs-CRP of patients treated with CAS tended to correlate with d-ROMs. To avoid ischemic complications, the value of the d-ROMs was meaningful, but not that of CRP.

Antioxidant potential and ageing

In rodent studies, antioxidant potentials were decreased in all organs with age. With ageing, the immune cells show an increase in oxidant and inflammatory compounds and a decrease in antioxidant defences, which is more evident in phagocytic cells. In our results, the antioxidant potential of patients treated by CAS decreased with ageing. We also showed the possibility of using d-ROM and BAP for the development of treatment strategies for cognitive function in elderly patients.

High oxidative stress correlates negatively with cognitive function

We showed that preoperative high oxidative stress influences cognitive function in patients undergoing CAS. In rodent experiments, cerebral hyperperfusion induced hydroxyl radicals and spatial memory deficits were induced several months afterwards. Hypoxia also decreased the expression of antioxidant mRNAs in rodents. In a human clinical study, high-intensity transient signals (HITS) occurred in asymptomatic low-grade carotid stenosis and HITS induced significant neuropsychological changes. Miyamoto et al reported that oxidative stress interferes with white matter repair by disrupting the renewal mechanisms in oligodendrocyte precursor cells. Radical scavengers may provide a potential therapeutic approach for white matter injury in vascular dementia and stroke. High oxidative stress produced by advanced atherosclerosis has the ability to influence cognitive function.

Oxidative stress during the CAS procedure

Temporal d-ROM values after extracorporeal circulation cardio-surgery were reported by Kanaoka et al to be increased for a duration of 2 weeks. However, they did not perform measurements during surgery or just after re-circulation. Our report is the first to show a temporal change in oxidative stress during carotid surgery.

Edaravone is a free radical scavenger, and the intraoperative application of this medication prevents hyperperfusion syndrome and ischemic complications. Sun et al showed that prophylactic edaravone prevents transient hypoxic ischemic brain injury in the mouse model, while delayed application (3 hours later) did not show any prevention of ischemia. We routinely use edaravone just before stenting to prevent these complications. Our results are the first to show that edaravone reduces oxidative stress significantly during CAS.

Additionally, post-stenting angioplasty increases oxidative stress in the carotid artery. Oxidative stress damages the endothelium, mitochondrial function, and neurons. Abe et al found that a single post-angioplasty local blood sample of interleukin 6 was higher in local lesions than in the aorta. This elevation of interleukin 6 was related to a new peri-procedural ischemic lesion. The blockade of radicals by distal balloon protection and the suction removal of blood after angioplasty prevented the harmful effects on the cerebral arteries and brain tissue.
High oxidative stress correlates with post-CAS ischemic lesions

During the CAS procedure, plaque scraped by devices, incomplete suction of debris, thrombus formation, and long occlusion times can all induce ischemic complications. Additionally, oxidative stress correlates with systemic atherosclerosis, which includes plaque deposition in the arteries. High oxidative stress means a high sclerotic state. A preoperative value for oxidative stress can alert surgeons to possible postoperative ischemic complications.

Limitations of this study

The number of participating patients was small and the sample was entirely Asian. There are many types of measurements of oxidative stress and antioxidant potential. A multicenter study with a larger, more diverse patient population is necessary to confirm these findings.

CONCLUSION

Preoperative oxidative stress correlates negatively with cognitive function and positively with ischemic lesions. High oxidative stress is a risk factor in ischemic complications with CAS. We also showed the possibility of using d-ROM and BAP values to develop a treatment strategy for elderly patients.

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Contributors

NS: Conception or design of the work, drafting the article, critical revision of the article, final approval of the version to be published. MN, TF: Data collection, data analysis and interpretation. NM, KoK, KiK, TF: NF: Data collection. HO: Critical revision of the article and final approval of the version to be published.

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Competing interests

None declared.

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Obtained.

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