

Advanced Glycation End Products in Patients with Cerebral Infarction

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Abstract

Objective Oxidative modification of carbohydrates and lipids enhances the formation of advanced glycation end products (AGEs), which are formed not only in hyperglycemia, but also in normoglycemia. In this study, we determined skin AGEs in patients with cerebral infarction.

Patients and Methods We non-invasively measured skin autofluorescence (AF) levels in patients with chronic cerebral infarction (CCI; n=95), patients with silent brain infarction (SBI; n=40), and age-matched controls (n=34), using an AGE Reader.

Results Skin AF levels in patients with CCI and SBI were significantly increased compared with those in the control group (2.06 ± 0.38 , 2.16 ± 0.47 and 1.84 ± 0.35 , respectively). Angiotension receptor blocker (ARB) or statins had no significant effect on the level of advanced glycation in any of the groups.

Conclusion Our data suggest that increased formation of AGEs may be an indicator of oxidative stress, not only in diabetes and renal failure, but also in chronic cerebral ischemia.

Key words: advanced glycation end products, AGE reader, cerebral infarction, oxidative stress

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Introduction

Oxidative modification of carbohydrates and lipids enhances the formation of reactive carbonyl species, which are capable of irreversibly transforming proteins to highly stable compounds that are generally referred to as advanced glycation end products (AGEs) (1). AGEs develop through complex, sequential reactions, collectively called the Maillard reaction (2). The accumulation of AGE results from a combination of hyperglycemia, oxidative/carbonyl stress, and/or decreased renal clearance of AGE precursors (2).

Accumulation of AGEs in tissues has been implicated in the progression of chronic, age-related diseases, such as atherosclerosis, chronic renal failure and diabetes mellitus (3). More recently, these compounds have also been shown to play a pivotal role in acute and chronic atherosclerotic disease, causing structural protein changes in the vascular wall, as well as activating cellular receptors, such as the receptor for AGEs, which in turn leads to activation of several

oxidative and inflammatory pathways (4-6).

Little is known about the implications of AGEs in relation to cerebral ischemia, although it has been established that AGE-modified proteins or peptides are neurotoxic factors that increase the volume of permanent damage and necrosis following focal cerebral ischemia (7).

Recently, a non-invasive method for measuring skin AGEs was developed by using the AGE Reader, which was introduced by Meerwaldt et al (3). This device permits rapid and non-invasive evaluation of AGEs by measuring the autofluorescence (AF) emitted from human skin. The method has been validated for specific AGEs measured in human skin biopsy samples from several patient groups and healthy controls. We hypothesized that skin AF is elevated in patients with asymptomatic as well as symptomatic cerebral infarction, because the accumulation of AGEs may reflect profound oxidative stress throughout the body. To test this hypothesis, we examined autofluorescence levels in the skin of patients with chronic cerebral infarction (CCI), patients with silent brain infarction (SBI), and age-matched controls, us-

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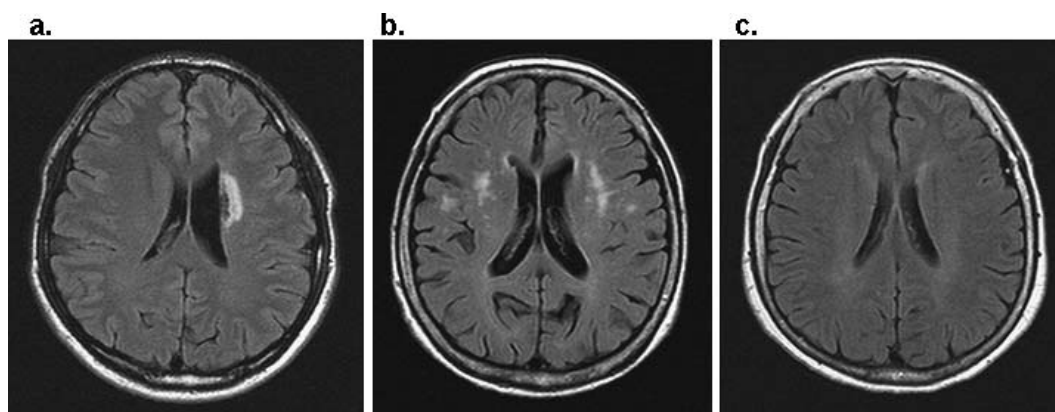


Figure 1. MRI FLAIR images of typical patients with a) chronic cerebral infarction, b) silent brain infarction and c) control.

ing an AGE Reader (DiagnOptics, The Netherlands). We also examined the effect of angiotensin receptor blocker (ARB) or statins on the level of advanced glycation in the CCI group.

Patients and Methods

Subjects

This study was performed between August 2006 and February 2007, at our Neurology Clinic in Tokai University Hospital. All patients were Japanese.

Ninety-five patients, who had suffered cerebral infarction at least 1 month before, were registered as the chronic cerebral infarction group (CCI) (Fig. 1a). Forty patients, who showed multiple infarcted areas accompanied with ischemic white matter changes on MRI but lacked neurological symptoms, were registered as the silent brain infarction group (SBI) (Fig. 1b). The NINDS criteria (8) were used in selection. We also checked the skin AGE level of age-matched control patients ($n=34$), who had complained of headache or dizziness and visited the Neurology Clinic in Tokai University Hospital, but showed no abnormal findings on MRI (Fig. 1c).

We excluded patients with diabetes and/or renal dysfunction who were over the upper normal limit of serum HbA1c (5.8%) or creatinine ($1.2 \mu\text{mol/L}$) from all 3 groups.

Skin autofluorescence

Skin AF was assessed on the ventral side of the right lower arm with an AGE reader, which consists of a tabletop box equipped with a black light excitation source. Excitation light in the range of 300 to 420 nm is projected onto the skin surface through a one square centimeter hole. The intensity of light reflected from the skin is measured between 420 to 600 nm with a spectrometer via a $200 \mu\text{m}$ glass fiber. Autofluorescence was calculated from the ratio of the intensities of the reflected light and the excitation light. The equipment was kept at the same location throughout the study, and the arm of each subject was covered with a black

cloth in order to avoid any influence of external light during the measurement.

Statistical analysis

Statistical analysis was done by ANOVA with the post hoc Turkey's test for comparison between groups in Figs. 2-4 using SPSS version 15.0J (SPSS Inc., Chicago, IL, USA). Two-tailed $p<0.05$ was considered significant. Data are shown as mean (\pm SD), unless otherwise indicated.

Results

Table 1 summarizes the characteristics of the 3 groups including age, sex, and medication. There was no difference in factors such as ARB and statin medication, which may influence the AGE levels, among the groups. Further, total cholesterol, triglyceride and blood pressure were not significantly different among the groups (data not shown).

Figure 2 shows the AGE levels in the 3 groups. Skin AGE levels in patients with CCI and those with SBI were significantly increased compared with the control group (2.06 ± 0.38 , 2.16 ± 0.47 and 1.84 ± 0.35 , respectively, $p<0.05$). However, there was no significant difference in AGEs between the CCI and the SBI groups.

Figure 3 shows the comparison among the stroke subtypes of the CCI group, including lacunar infarction ($n=47$), atherothrombotic ($n=24$), cardioembolic infarction ($n=19$) and others ($n=5$). The AGE levels were not significantly different among these groups (2.07 ± 0.39 , 1.95 ± 0.42 , 2.11 ± 0.33 , 2.14 ± 0.33 , respectively, $p>0.05$).

To clarify the effect of medication on the skin AGE levels, the differences in AGE levels in subgroups of the CCI group treated with either statin ($n=10$), or ARB ($n=30$), or both ($n=4$), and neither ($n=51$) were reanalyzed (Fig. 4). However, there were no significant differences among the 4 subgroups, although the ARB+statins-treated subgroup showed a tendency for the AGE level to be lower than in the other subgroups.

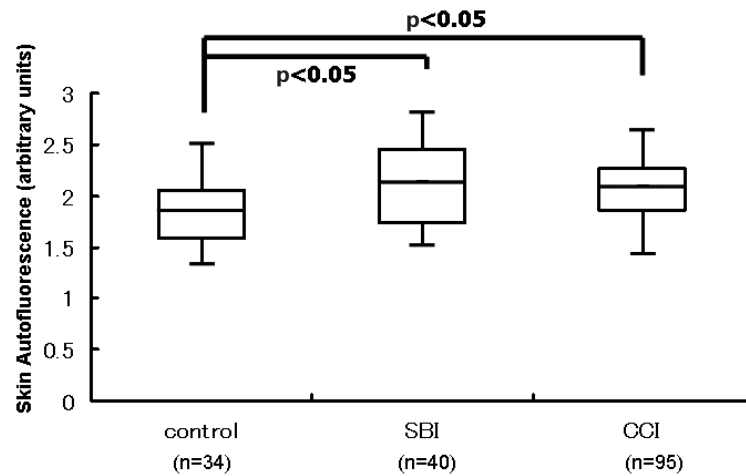


Figure 2. Skin AGEs levels measured with the AGE reader in the 3 groups. Skin AGEs levels in patients with chronic cerebral infarction (CCI)(n=95) and silent brain infarction (SBI)(n=40) were significantly increased compared with the control group (n=34)($p<0.05$). There was no significant difference in AGEs between the CCI and SBI groups. The horizontal line within a box represents the median, and the lower and upper end of a box are the first and third quartile, respectively. The lower line and the upper line outside the box represent the 5th and 95th percentile, respectively.

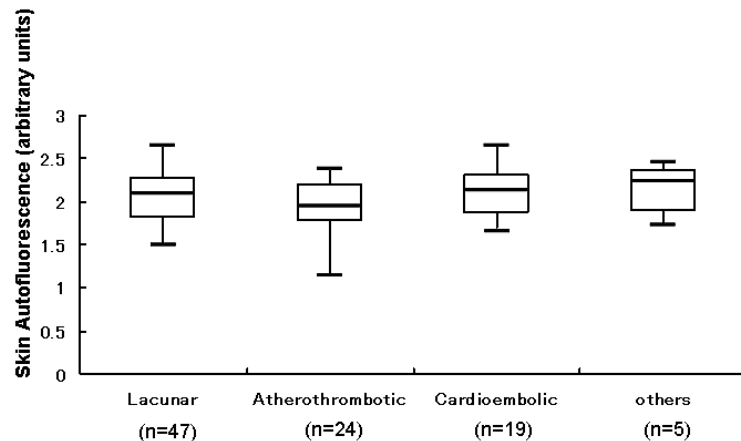


Figure 3. Comparison of AGEs levels between subtypes in the CCI group. There were no significant differences in AGEs levels in the chronic cerebral infarction (CCI) group among the subtypes; lacunar (n=47), atherothrombotic (n=24), cardioembolic infarction (n=19) and others (n=5).

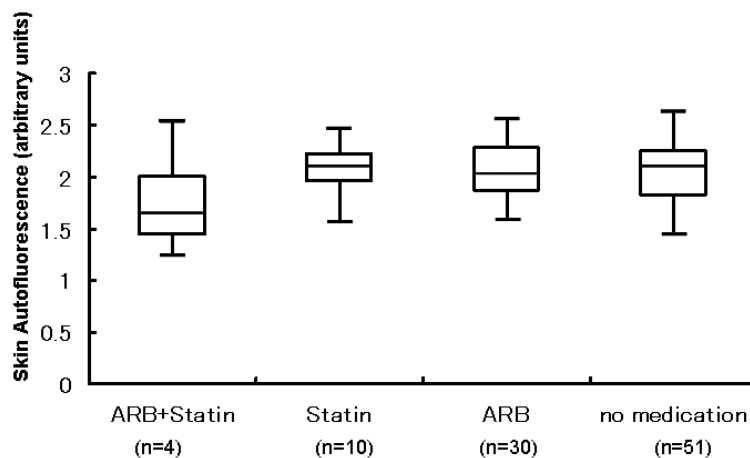


Figure 4. Effect of medication on skin AGEs levels in the CCI group. There were no significant differences in AGE levels in the chronic cerebral infarction (CCI) group among the subgroups taking statin (n=9), or ARB (n=30), or both (n=5), or neither (n=51), although the ARB+statin-treated group tended to show a lower AGEs level than the others.

Table 1. Characteristics in the 3 Groups Studied

	Age-matched Control (n=34)	Silent brain infarction (n=40)	Chronic infarction (n=95)
Ages (years)	68±6	71±9	68±10
Blood Pressure (mmHg)	130±14 / 72±10	137±17 / 74±10	135±14 / 75±9
Sex (M:F) (n)	16 : 18	18 : 22	63 : 32
Medications:			
Statin + ARB (n)	0	1	4
Statin (n)	4	5	10
ARB (n)	4	6	30
No medication (n)	26	29	51

Table 1. Characteristics of patients in the 3 groups. This table shows the characteristics of subjects enrolled in the age-matched control, the silent brain infarction and chronic cerebral infarction groups. Patients with diabetes and/or renal dysfunction (serum HbA1c over 5.8% or creatinine level over 1.2 μ mol/l) were excluded. All patients were Japanese. There was no significant difference of blood pressure among the 3 groups.

Discussion

This is the first report of non-invasive measurement of AGEs in patients with cerebral infarction, but without diabetes or renal failure. The pathogenesis of cerebral infarction is associated with oxidative stress, which increases the formation of stable AGEs. Skin AF is thought to serve as a non-invasive measure of hyperglycemia and inflammation-derived oxidative stress (1). It has also been reported that increased skin AF reflects increased AGE accumulation (3). Moreover, some non-fluorescent AGEs, such as N^ε-(carboxymethyl)lysine (CML), are thought to play an important pathogenic role. Skin AF was shown to be correlated with skin levels of CML (2). Thus, skin AF may be a marker of total skin AGE accumulation.

Skin AF is also known to be a risk factor for long-term diabetic complications, such as impaired glycemic control (3). AGEs play a role in the pathogenesis of several other diseases, such as renal failure, atherosclerosis and Alzheimer's disease (2, 3). However, the relation between AGEs and cerebral infarction is poorly understood. Zimmerman et al showed that systemically administered AGEs were markedly neurotoxic, as evidenced by significantly larger infarct volumes in AGE-treated animals. They concluded that AGEs themselves might contribute to neurotoxicity associated with diabetes and renal failure (7).

We believe that the AGE Reader provides a new approach to predict recurrent ischemic events. However, there are several limitations to the present study. First, the skin color of patients might affect the measured AF values, and the AGE Reader is not reliable for dark brown skin. In most previous studies, the subjects were Caucasian (2, 3), but some reports have presented AF in Japanese patients with rheumatoid arthritis, osteoarthritis and dialysis-related spondyloarthropathy (14), and end-stage renal disease (15). Second, the detection

of AF by the AGE Reader is influenced by circumstances such as the position of the arm, room temperature and external light. In order to minimize these influences, we kept the equipment at the same location, and the same side of the arm in each subject was covered with a black cloth. Third, the AF values showed such high a variation in each disease that more patients should be included in a larger study to enable better statistical analysis.

The skin AGE levels in patients with CCI and SBI were significantly increased compared with the control group. Furthermore, AGEs in the CCI and SBI groups were not significantly different. These results suggest that asymptomatic cerebral infarction is associated with oxidative stress. We hypothesized that ACI might be the prodromal state of CCI, because several studies have shown that ACI is an independent predictor of the risk of symptomatic stroke (9-11), and it was suggested that AGE accumulation was associated with injury to the endothelium.

In addition, we observed an elevation of skin AGEs in patients with all types of cerebral infarction, even though we had excluded patients with diabetes and/or renal dysfunction. We could not observe any significant difference of AGE levels between the cardioembolic and atherothrombotic infarction groups, but AGE elevation may be considered to reflect whole-body oxidative stress, which causes atrial fibrillation in the heart (12, 13).

We also tested the effect of medications including either a statin, or ARB, or both, or neither on AGEs in patients with chronic infarction. Miyata et al (16) reported that olmesartan could decrease proteinuria in contrast with calcium channel blocker and beta blocker, and ARBs have unique renoprotective properties, including the inhibition of rennin-angiotensin system (RAS), oxidative stress, AGEs. On the other hand, Jinnouchi et al (17) reported that atorvastatin decreases serum levels of AGEs in type 2 diabetic patients. We hypothesized that ARBs or statin could decrease AF value in the pa-

tients with cerebral infarction measured with AGE Reader. However, there were no significant differences among these subgroups, though a tendency for reduced AGEs was seen in patients receiving both ARB and statin. The reason for this is unclear, but differences in duration, dose and kind of medication might influence AGEs values.

In conclusion, the AGE Reader is a non-invasive, semiquantitative, and convenient tool for the measurement

of AGE levels in out-patient clinics. Hankey et al (18) proposed 55 potential new risk factors for ischemic stroke. Our data suggest that increased formation of AGEs may be one of the indicators of oxidative stress, not only in diabetes and renal failure, but also in chronic cerebral ischemia. Further study will be required to establish whether skin AGEs level is a useful parameter for predicting recurrence of ischemic stroke.

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