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REVIEW

Protein-conjugated acrolein as a biochemical marker of brain infarction

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The relationship between acrolein (CH_2=CH-CHO) and brain infarction is the focus of this review. It has been found that acrolein is produced mainly within cells from polyamines by polyamine oxidases (PAOs), especially from spermine by spermine oxidase during cell damage, and that acrolein is more toxic than reactive oxygen species (ROS) in a cell culture system. Thus, the possibility that acrolein and PAOs are good biochemical markers of stroke was tested because there are no other reliable biochemical markers at the early stage of stroke. Levels of protein-conjugated acrolein (PC-Acro) and PAOs (acrolein-producing enzymes) were significantly increased in the plasma of stroke patients. The multiplied value of PC-Acro by PAOs was nearly parallel with the size of stroke. Furthermore, when the combined measurements of PC-Acro, interleukin-6 (IL-6) and C-reactive protein (CRP) were evaluated along with age using a receiver operating characteristic (ROC) curve, even silent brain infarction (SBI), which is a small brain infarction, was indicated with approximately 84% sensitivity and specificity. These findings clearly indicate that acrolein is strongly correlated with cell damage during brain infarction.

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1 Introduction

Oxidative stress, which can lead to various disorders, is thought to be caused by two kinds of compounds – first, reactive oxygen species (ROS) such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) [1, 2], and second, unsaturated aldehydes such as acrolein [3, 4]. In this review, we present evidence that acrolein is involved more prominently than ROS in some forms of cell damage, in contrast to prevailing theories that ROS plays a predominant role in cell damage.

Polyamines (putrescine, spermidine (SPD) and spermine (SPM)) are necessary for the normal cell growth and are present at millimolar concentrations in cells [5, 6]. Polyamine content in cells is tightly regulated by biosynthesis, degradation and transport [7, 8]. However, the addition of SPD or SPM to culture medium containing ruminant serum is known to inhibit cellular proliferation [9]. This effect is caused by the oxidation products of SPD and SPM that are generated by ruminant serum amine oxidase (Fig. 1A) [10]. This enzyme produces both H_2O_2 and acrolein. Thus, we compared the toxicity of H_2O_2 and acrolein on mouse mammary carcinoma FM3A cells. Growth inhibition caused by SPM was recovered by aldehyde dehydrogenase but not by catalase, and 15 μM acrolein and 200 μM H_2O_2 inhibited cell growth to almost the same degree (Fig. 1B) [11]. The results indicate that acrolein is the

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Abbreviations: AcPAO, acetylpolyamine oxidase; **CA,** carotid atherosclerosis; **CRP,** C-reactive protein; **CT,** computed tomography; **DSWMH,** deep and subcortical white matter hyperintensity; **FDP-lysine,** *N*-(3-formyl-3,4-dehydropiperidino)-lysine; **HNE,** 4-hydroxy-2-nonenal; **MRI,** magnetic resonance imaging; **8-OHdG,** 8-hydroxy-2′-deoxyguanosine; **PVH,** periventricular hyperintensity; **PAO,** polyamine oxidases; **pSS,** primary Sjögren's syndrome; **PC-Acro,** protein-conjugated acrolein; **PC-HNE,** protein-conjugated 4-hydroxy-2-nonenal; **PIT,** photochemically induced thrombosis; **ROC,** receiver operating characteristic; **ROS,** reactive oxygen species; **RRV,** relative risk value; **SBI,** silent brain infarction; **SMO,** spermine oxidase; **SPD,** spermidine; **SPM,** spermine; **SSAT,** spermidine/spermine N^1-acetyltransferase; **WMH,** white matter hyperintensity

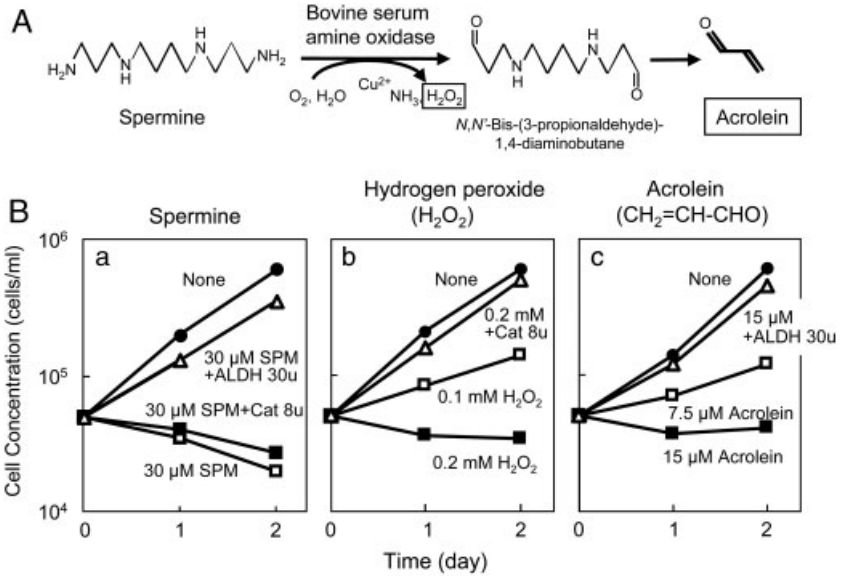

Figure 1. Effect of SPM, H_2O_2 and acrolein on cell growth of mouse mammary carcinoma FM3A cells[a)]. (A) Degradation of SPM by bovine serum amine oxidase. (B) Cell growth of FM3A cells. Chemicals and enzymes (catalase (Cat)) and aldehyde dehydrogenase (ALDH) were added to the medium along with 2% fetal calf serum. [a)] Data adapted from [11].

major toxic compound produced from SPM by amine oxidase [11]. In addition, it was found in another experiments that complete inhibition of cell growth was accomplished with 10 μM acrolein, 100 μM H_2O_2 and 20 μM hydroxyl radical (OH) produced by 20 μM H_2O_2 and 1 mM vitamin C [12]. Based on these findings, it was tested whether acrolein and acrolein-producing enzymes, i.e. polyamine oxidases (PAOs), are good biochemical markers for cell damage in conditions such as brain infarction.

2 Protein-conjugated acrolein (PC-Acro) and PAOs in plasma as biochemical markers of brain stroke

Stroke is a sudden focal neurological deficit caused by vascular insult, accompanied by cell damage in the central nervous system. At present, there is no reliable biochemical marker for diagnosis of the early stage of stroke. To look for biochemical markers for stroke, the levels of PC-Acro and PAOs in plasma of stroke patients were measured. Since acrolein easily reacts with lysine residues of proteins [13–15], PC-Acro was measured by enzyme-linked immunosorbent assay (ELISA) using anti-Nε-(3-formyl-3,4-dehydropiperidino)-lysine (FDP-lysine) antibody [16] instead of free acrolein. Focal infarcts were estimated by magnetic resonance imaging (MRI) and computed tomography (CT).

SPM is metabolized via two pathways: one involves conversion to SPD and 3-aminopropanal by spermine oxidase (SMO), and the other involves metabolism to SPD and 3-acetamidopropanal by spermidine/spermine N^1-acetyltransferase (SSAT) and acetylpolyamine oxidase (AcPAO) (Fig. 2A) [17, 18]. We reported that acrolein is mainly produced from 3-aminopropanal, which is formed from SPM by SMO, but is also produced from 3-acetamidopropanal, which is formed from SPM and SPD by SSAT and AcPAO [11, 19]. Accordingly, the activities of SMO and AcPAO were measured along with the level of PC-Acro (FDP-lysine) in the plasma of patients with stroke. SMO and AcPAO were measured enzymatically using SPM and N^1-acetylspermine as substrate, respectively [20]. The levels of AcPAO, SMO, total polyamine oxidases (PAO; AcPAO plus SMO) and PC-Acro were significantly higher in the plasma of patients with stroke (Fig. 2B). The median levels of AcPAO, SMO, PAO and PC-Acro in patients with stroke compared with the control subjects increased from 0.9 to 3.1, from 3.2 to 4.7, from 4.5 to 8.0 and from 14.4 to 21.3 nmol/mL plasma, respectively. When we analyzed the level of polyamines in plasma from 12 patients from day 1 to day 20 after the onset of stroke, there was a tendency for the level of putrescine to be increased, whereas levels of SPM and SPD were significantly decreased. The results support the idea that AcPAO and SMO are released from nerve, glia or other cells during the early period of stroke, leading to reduced levels of SPM and SPD and an increased level of PC-Acro (FDP-lysine). It has been reported that acrolein is produced mainly from membrane phospholipids, although the major aldehydes produced during lipid peroxidation are 4-hydroxy-2-nonenal (HNE) and malondialdehyde [21]. We measured acrolein produced from arachidonic acid under the same conditions in which acrolein has been reported to be produced from membrane phoshpolipids [16]. However, acrolein production was very low [20]. The proposed mechanism of inefficient formation of acrolein from arachidonic acid [22] and experimental results on very low production of acrolein from arachidonic acid [23] also support the idea that acrolein is mainly produced from polyamines, but not from unsaturated fatty acids by lipid peroxidation. Thus, we believe that acrolein is produced mainly from SPM rather than from unsaturated fatty acids.

We then examined whether the increases in PAO and PC-Acro are correlated with the severity of stroke (size of the

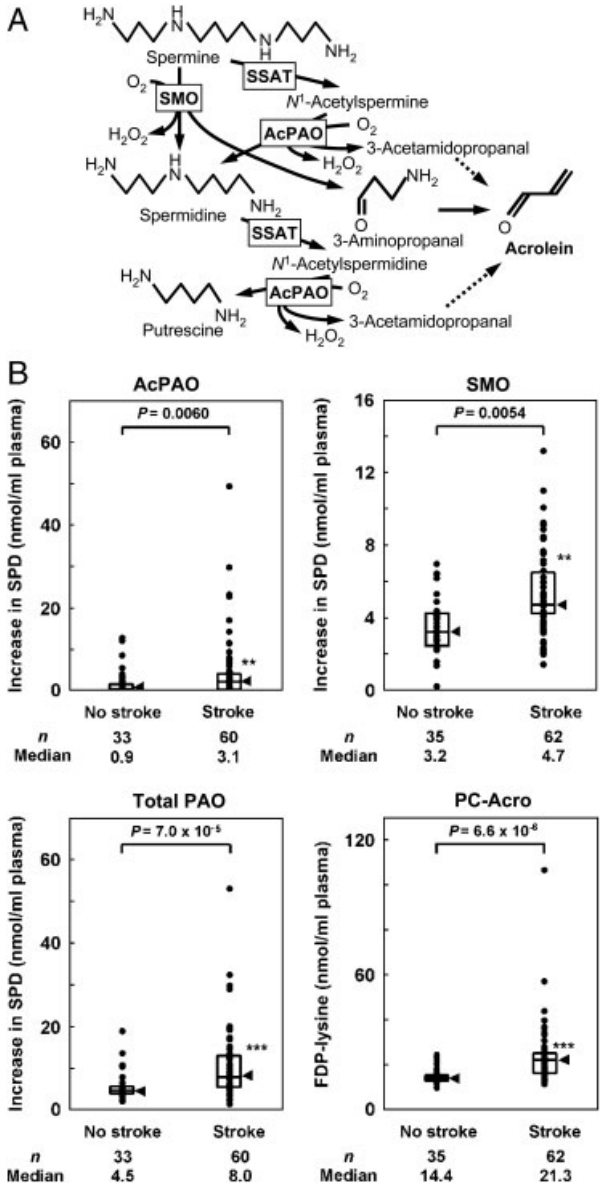

Figure 2. Levels of AcPAO, SMO, total PAO, and PC-Acro in the plasma of patients with brain stroke[a)]. (A) Acrolein productions from SPM and SPD. AcPAO, acetylpolyamine oxidase; SMO, spermine oxidase; SSAT, spermidine/spermine N^1-acetyltransferase. (B) Activities of AcPAO, SMO, total PAO (AcPAO plus SMO) and level of PC-Acro in plasma were compared with no-stroke subjects and patients with brain stroke. Values are shown in median (arrowhead) ± interquartile deviation shown as box. [a)] Data adapted from [20].

infarct) from day 1 to day 20 after the onset of stroke with 16 patients in this time window. Because the maximal increase in PAO precedes that in PC-Acro, and the increase in PC-Acro is dependent on the changes in PAO, the multiplied value of PC-Acro by the total PAO was compared with the size of the infarct. Statistical significance between stroke patients with no-stroke subjects became greater in the multiplied value ($p = 9.3 \times 10^{-7}$) than PC-Acro ($p = 6.6 \times 10^{-6}$) or PAO

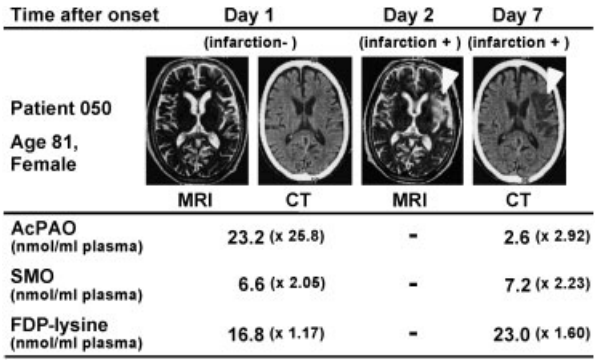

Figure 3. Relationship between imaging (T2-weighted MRI and CT) and biochemical markers (AcPAO, SMO, and PC-Acro (FDP-lysine))[a)]. Time-dependent alteration of imaging and biochemical markers in plasma was examined in one patient with brain stroke. Arrowheads indicate the position of focal infarcts. Values are shown along with the degree of increase compared with no-stroke subjects in the parentheses. [a)] Data adapted from [20].

($p = 7.0 \times 10^{-5}$). The size of the infarct was nearly parallel with the multiplied value of PC-Acro by PAO [20].

There was also a patient who came to our hospital with a suspected stroke (Fig. 3). On day 1 (within 6 h after the onset of stroke), the levels of AcPAO and SMO were elevated (25.8- and 2.05-fold higher than control), along with a small increase in PC-Acro (FDP-lysine) (1.17-fold). At that time, focal infarcts were not observed either MRI (T2-weighted MRI) or CT. On day 2, a large infarct was clearly observed at the left temporal lobe by MRI (T2-weighted MRI). On day 7, the levels of AcPAO, SMO and PC-Acro (FDP-lysine) were still elevated, and infarction was clearly observed by CT. Thus, the increase in AcPAO and SMO in plasma was the very early diagnostic marker to confirm stroke in this patient.

The results from these clinical studies are also paralleled by animal studies using rat models of cellular injury. SSAT and SMO are induced during kidney ischemia-reperfusion injury in rats [24], and SPM and SPD decreased after transient focal cerebral ischemia in spontaneously hypertensive rats [25]. 3-Aminopropanal, which automatically produces acrolein, is generated from SPM and is strongly involved in cell damage during ischemia in rats [26, 27]. The level of polyamines increased in aorta and ventricular tissues when hypertension is induced by angiotensin II in rats [28]. Thus, acrolein may be produced in patients who suffer stroke as a consequence of untreated hypertension. We also observed that the levels of PAO and PC-Acro increased in hemorrhagic stroke, although the number of patients was only 6.

3 Correlation between brain infarction and PC-Acro in photochemically induced thrombosis (PIT) model mice

It was determined whether acrolein is indeed produced from SPM and SPD at the locus of infarction using a mouse

model of stroke based on photochemically induced thrombosis (PIT) [29]. A unilateral infarction was induced in mouse brain by photoinduction after injection of Rose Bengal [30], and the volume of the infarct was determined by staining 2-mm-thick coronal slices with triphenyltetrazolium. This stains the viable brain tissue red, whereas infarct tissue remains unstained [31]. Under our experimental conditions, the average volume of infarction at 24 h after photoinduction was 23 mm^3 (Fig. 4A). PC-Acro (68 kDa) at the locus of the brain infarction was 28-fold higher than that at the same locus of the control mice (Fig. 4A). The protein was also stained with an antibody against albumin (Fig. 4A), indicating that most of PC-Acro is albumin. In this respect, it has been reported that ischemia-modified albumin levels are high in cerebrovascular diseases [32].

We also measured the level of polyamines at the locus of infarction to confirm that acrolein is produced mainly from SPM and SPD. Levels of both SPD and SPM decreased significantly after the infarction, whereas the level of putrescine increased (Fig. 4A). The results strongly suggest that acrolein is produced during the conversion of SPM into SPD, and SPD to putrescine by SMO, SSAT and AcPAO. Indeed, the size of the infarct decreased significantly when 100 mg/kg of MDL72527 (*N,N′*-butanedienyl butanediamine), an inhibitor of PAOs [33], was administered intraperitoneally prior to the onset of brain infarction [19]. Levels of PC-Acro, putrescine and SPD in plasma were significantly higher in PIT model mice than in the control mice (Fig. 4B). Activities of SMO and AcPAO, which are sensitive to MDL72527, were also significantly higher in the plasma of PIT model mice than the control mice (Fig. 4B). These results confirm that PC-Acro, polyamines and PAOs were released from the locus of infarction.

We found that *N*-acetylcysteine is a strong acrolein scavenger rather than an ROS scavenger [12], although it is often considered as an ROS scavenger [34]. Thus, in a cell culture system, *N*-acetylcysteine markedly attenuated the inhibition of cell growth induced by acrolein, but it had modest effects on growth inhibition produced by hydroxyl radical (OH) and no effect on growth inhibition produced by H_2O_2 [12]. In case of *N*-acetyllysine, it took a longer time to scavenge acrolein compared with *N*-acetylcysteine [12]. Glutathione did not pass through blood–brain barrier. Accordingly, the effects of *N*-acetylcysteine on brain infarction were examined to confirm the correlation between brain infarction and acrolein [29]. At 24 h after induction of infarction, the average volume of infarction decreased from 23 to 16 mm^3 in PIT model mice. PC-Acro at the locus of infarction greatly decreased, and levels of polyamines increased significantly by the injection of *N*-acetylcysteine. These results again suggest that acrolein is effectively scavenged by *N*-acetylcysteine.

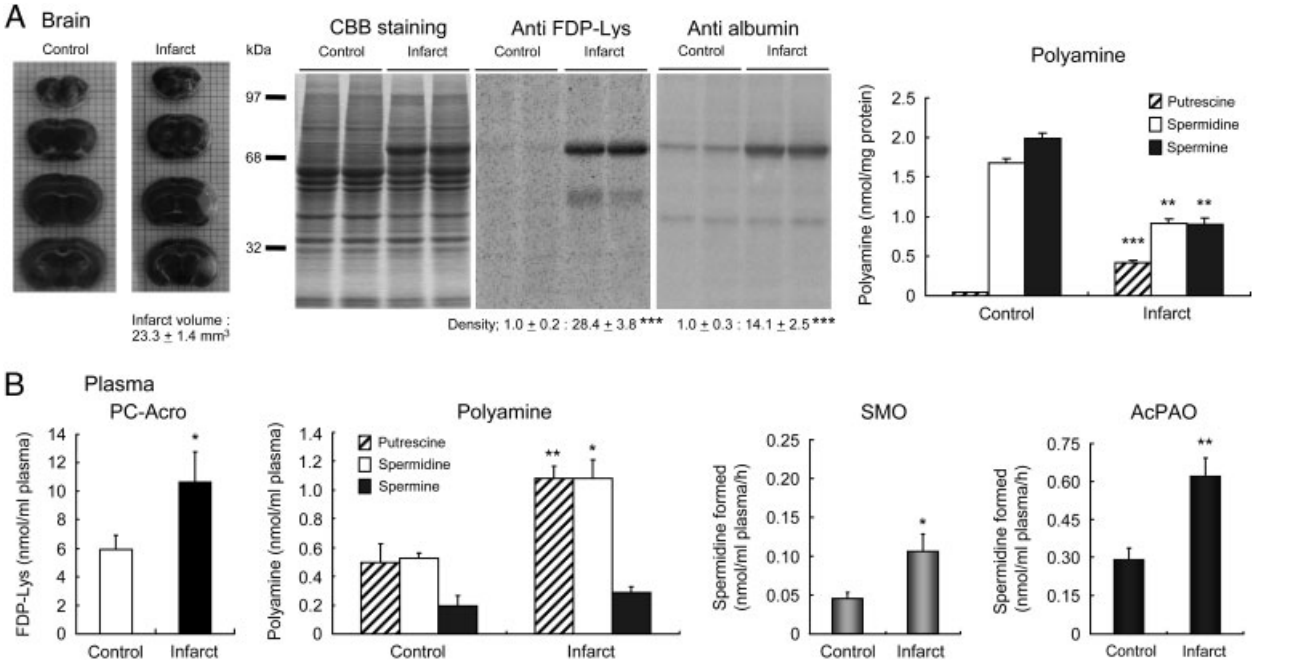

Figure 4. Correlation between brain infarction and PC-Acro in PIT model mice[a)]. (A) Infarction volume at 24 h after the induction of infarction, the levels of PC-Acro and albumin estimated by Western blotting, and polyamines at the locus of brain infarction and at the corresponding locus in normal mice are shown. (B) Increase in PC-Acro, polyamines and PAOs in plasma by brain infarction is shown. Polyamines (putrescine, SPD and SPM) were measured by fluorescence produced with *o*-phthalaldehyde after separation of polyamines by HPLC [58]. $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. [a)] Data adapted from [29].

4 Intense correlation between brain infarction and acrolein rather than ROS

We subsequently compared the relative participation of acrolein and ROS in cell damage in brain infarction using PIT model mice [19]. As indicators of cell damage, levels of protein, DNA, RNA and phospholipid at the locus of infarction were compared with levels at the corresponding locus in the control mice (Fig. 5A). Although the content of all macromolecules (protein, DNA, RNA and phospholipid) at the locus of infarction was low compared with that in the normal mice, the level of RNA was drastically decreased. The results suggest that RNA breakdown is a potential initiator of cell damage. The degree of cell damage produced by acrolein and ROS was then compared. Both HNE produced from unsaturated fatty acid [22] and 8-hydroxy-2′-deoxyguanosine (8-OHdG) [35] were used as markers of oxidative damage caused by ROS. Although levels of protein-conjugated 4-hydroxy-2-nonenal (PC-HNE) and 8-OHdG at the locus of infarction were two to fourfold greater than

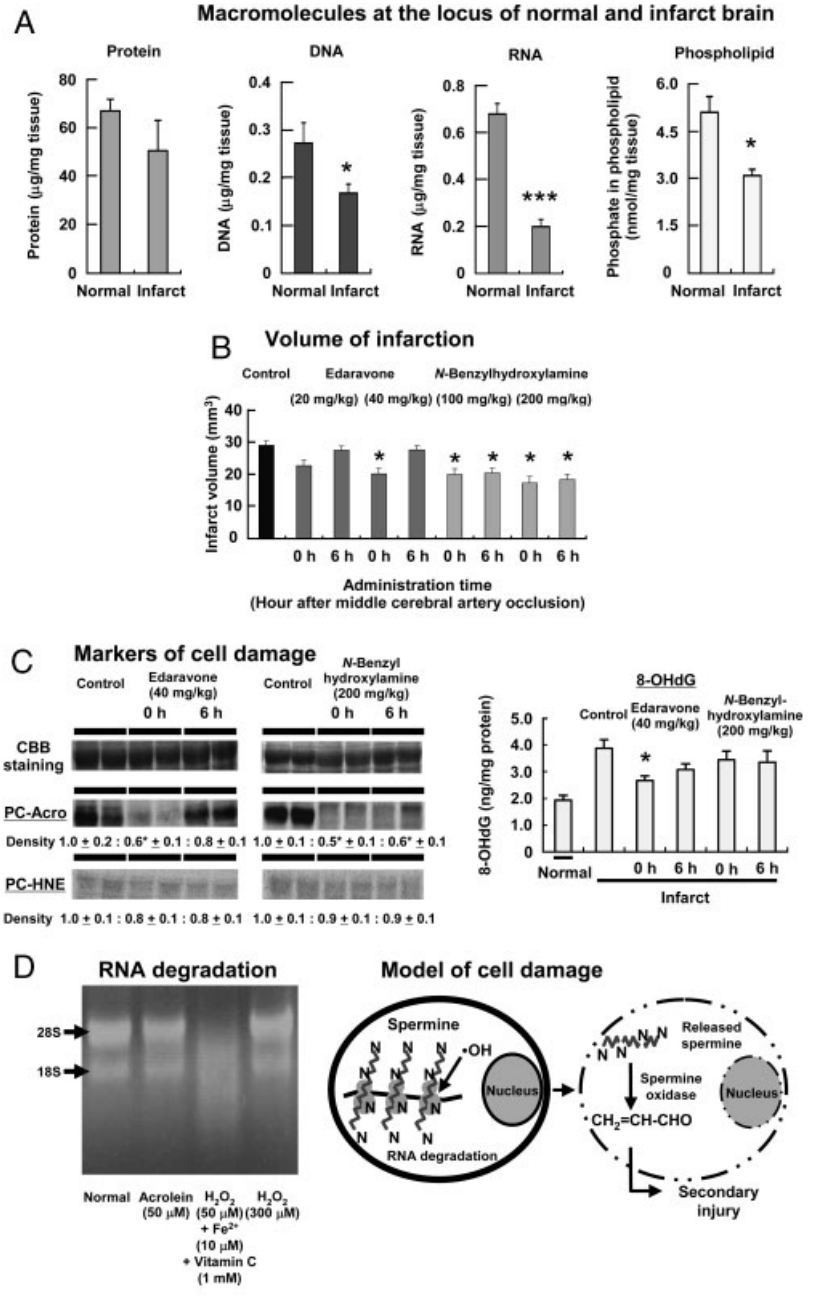

Figure 5. Increase in markers of cell damage during brain infarction and effect of edaravone and *N*-benzylhydroxylamine on brain infarction in mice[a)]. (A) The levels of protein, DNA, RNA and phospholipid at the locus of infarction and at the corresponding locus in normal mice are shown. (B, C) Infarction volume and markers of cell damage (PC-Acro, PC-HNE and 8-OHdG) are shown. (D) Effect of acrolein, OH produced from H_2O_2 in the presence of Fe^{2+} and vitamin C, and H_2O_2 on RNA degradation, and model of cell damage by acrolein and ROS are shown. $^{*}p<0.05$, $^{***}p<0.001$. [a)] Data adapted from [19].

levels at the corresponding locus in the normal mice, levels of PC-Acro were increased by 25- to 30-fold. The toxicity of acrolein was nearly equal to that of HNE in both suspension and monolayer culture. These results indicate that, in brain infarction, acrolein is involved more prominently than ROS in cell damage [19].

In blood, high concentrations of pyruvic acid and α-ketoglutaric acid exist. Those are strong ROS scavengers [12]. Thus, toxicity of ROS is strongly reduced in the presence of blood.

The effects of edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one), a scavenger of ROS [36], and *N*-benzylhydroxylamine, a scavenger of acrolein [37], on the extent of brain infarction were compared (Fig. 5B). Edaravone reduced the infarct volume when it was administered intraperitoneally prior to the onset of infarction (Fig. 5B), but not when it was administered 6 h after the onset of infarction, which confirmed the previous results [36]. In contrast, *N*-benzylhydroxylamine reduced the infarct volume when it was administered intraperitoneally either at the onset of infarction or 6 h later. These results indicate that ROS has effects only at the early period of brain infarction. When the infarct was reduced by edaravone, both PC-Acro and the marker of oxidative damage caused by ROS (PC-HNE and 8-OHdG) decreased (Fig. 5C), but the effect on PC-Acro was more pronounced than effects on PC-HNE and 8-OHdG. The results suggest that ROS induces acrolein production and acrolein is strongly involved in the progression of infarction. When the infarct was reduced by *N*-benzylhydroxylamine, only PC-Acro significantly decreased (Fig. 5C). Furthermore, when the infarct was reduced by edaravone and *N*-benzylhydroxylamine, the levels of polyamines increased, indicating that acrolein is produced mainly from polyamines.

We then studied how ROS induces acrolein production. Polyamines exist mainly as RNA–polyamine complex in cells [38]. Thus, it was determined whether ROS promotes the release of polyamines from RNA. It was found that OH, but not H_2O_2 and acrolein, caused RNA degradation and release of polyamines, leading to increased production of acrolein. These findings suggest that ROS are involved in the initiation of infarction, whereas acrolein, produced from released polyamines, is involved in the expansion of infarction (Fig. 5D) [19].

We confirmed that acrolein is produced mainly from SPM by SMO rather than from SPM and SPD by SSAT and AcPAO using SPM-less Gy mice that are deficient in spermine synthase and transglutaminase 2-knockout mice in which the activity of SSAT was elevated. Infarction volume caused by PIT in Gy mice was reduced compared with wild-type mice, although the level of SPD was elevated in Gy mice. Similarly, infarction volume caused by PIT in transglutaminase 2-knockout mice was reduced although the activity of SSAT was increased. The results clearly show that acrolein is produced mainly from SPM by SMO [19].

5 Detection of silent brain infarction (SBI) by PC-Acro, IL-6 and C-reactive protein (CRP) in plasma with high sensitivity and specificity

There are reports that SBI increases the risk of subsequent stroke [39–41], dementia [41] and mild cognitive impairment [42]. There is also a report that SBI is more common in patients with obstructive sleep apnea [43]. Furthermore, it has been reported that carotid atherosclerosis (CA) is a risk factor for stroke and SBI [44, 45], and that SBI and marked white matter hyperintensity (WMH) for stroke [46]. Thus, we tested how PC-Acro is correlated with SBI, WMH and CA by collecting blood from 790 elderly healthy volunteers. Since the levels of CRP and IL-6 are reported to increase in the serum of apparently healthy individuals with SBI [47], we measured the levels of CRP and IL-6 along with PC-Acro in plasma [48, 49]. SBI (affected areas $\geq$3 mm diameter) and WMH were estimated by MRI, CA by carotid ultrasound examination and CRP and IL-6 in plasma by ELISA.

The distribution of subjects as control (260 subjects), SBI (214 subjects), CA (263 subjects) and WMH (245 subjects) was classified as a function of age. Incidence of SBI and CA increased with age from 50th to over 70th, but the incidence of WMH was nearly equal from 50th to over 70th. The incidence of SBI increased from 12 to 54%, CA from 22 to 58%, but that of WMH was 31–36% from 50th to over 70th. The association of SBI with CA, SBI with WMH and WMH with CA was approximately 40, 6 and 37%, respectively. The results indicate that the association of SBI with WMH is small. PC-Acro, IL-6 and CRP were significantly higher in SBI and CA compared with the control. PC-Acro was most strongly associated with CA, and IL-6 and CRP with SBI [49]. Thus, CA is likely the primary risk factor related with stroke in this analysis.

We next determined whether SBI, CA and WMH could be detected by altered levels of PC-Acro, IL-6 and CRP using a receiver operating characteristic (ROC) curve – a commonly used technique for assessing diagnostic and predictive accuracy in disease management [50]. Since age was an important factor among various markers evaluated for the detection of SBI, CA and WMH, these three values were analyzed including age as a factor.

The ROC curve for the detection of SBI by age, with measurement of PC-Acro, IL-6 and CRP is shown in Fig. 6A. Sensitivity and specificity were 84.1 and 83.5%, respectively. Relative risk values (RRV) of brain infarction were then calculated for SBI, CA and WMH. A value of 1 is the highest value, and 0 is the lowest value as an index of the degree of tissue damage. The median RRV for SBI with CA (93 subjects), SBI (214 subjects), CA (263 subjects), WMH with CA (90 subjects), WMH (245 subjects) and control (260 subjects) was 0.90, 0.80, 0.76, 0.65, 0.46 and 0.14, respectively (Fig. 6B). In this case, SBI, CA and WMH include subjects with single, double and triple pathologies [49].

We also studied whether the median RRV is correlated with the severity of CA. The severity of CA was evaluated by

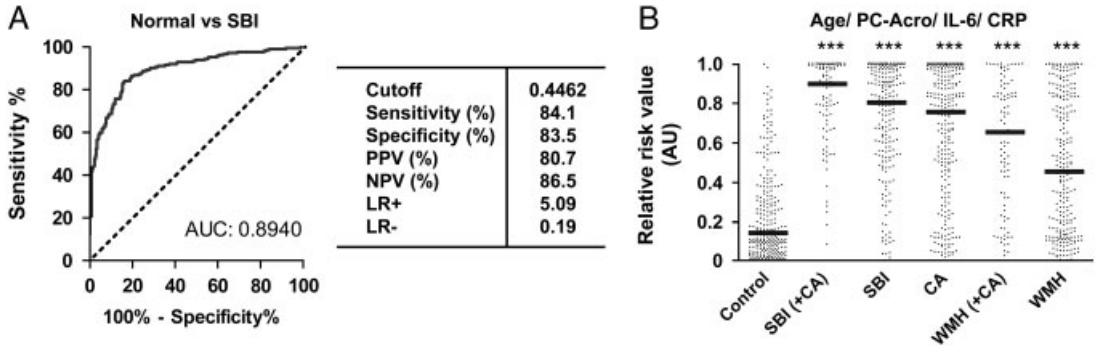

Figure 6. ROC curve of age/PC-Acro/IL-6/CRP for SBI versus control subjects (A) and relationship between relative risk value (RRV) and SBI, CA and WMH (B)[a)]. AUC, area under curve. RRV calculated from ROC curve is shown with median (horizontal line). ***$p<0.001$ compared with control subjects. [a)] Data adapted from [49].

the additive value of max-IMT (intima-medial thickness) in both right and left carotid arteries. Definitions of ''mild'' and ''severe'' are max-IMT values of 1.1–2.9 mm and $\geq$3 mm, respectively. Age and PC-Acro were significantly increased, and IL-6 and CRP were slightly higher in subjects with severe CA than those in mild CA. Accordingly, the median RRV of 175 mild subjects and 88 severe subjects was 0.67 and 0.90, respectively. With an increase in severity, from mild to severe, the association with SBI increased from 30 to 45% [49].

The correlation between the severity of WMH and the median RRV was also studied. WMH was subclassified into 166 subjects with a single periventricular hyperintensity (PVH) or deep and subcortical white matter hyperintensity (DSWMH) and 79 subjects with both PVH and DSWMH. Age and PC-Acro were significantly higher in subjects with both PVH and DSWMH than in subjects with a single PVH or DSWMH. Difference of IL-6 and CRP was not significant between two groups. Thus, RRV for the subjects with PVH or DSWMH and with both PVH and DSWMH was 0.34 and 0.55, respectively. With an increased severity of WMH, the association with SBI increased from 3.6 to 10% [49].

Although PC-Acro is well correlated with brain infarction, measurement of IL-6 and CRP along with PC-Acro was necessary to increase sensitivity and specificity to detect SBI. It has been reported recently that IL-6 is effectively produced by astrocytes for neuroprotection during brain infarction [51, 52]. Thus, it was reasonable to measure IL-6 to estimate the severity of SBI and brain infarction, and also to increase sensitivity and specificity of SBI.

It has been also reported that PC-Acro increases in patients with Alzheimer's disease [53] and in the patients with spinal cord injury [54].

6 PC-Acro in renal failure and Sjögren's syndrome

Since acrolein is a major toxic compound in cells and produced in all kinds of cells, we looked for a correlation between PC-Acro and other diseases involving tissue destruction, i.e. chronic renal failure and Sjögren's syndrome. In the case of chronic renal failure, a very strong correlation with PC-Acro was observed [13, 55]. Severity of chronic renal failure is classified by the level of creatinine, i.e. normal, $<$1.2 mg/100 mL plasma; moderate, $<$8 mg/100 mL plasma and severe, $\geq$8 mg/100 mL plasma. PC-Acro (FDP-Lys) of normal ($n=19$), moderate ($n=13$) and severe ($n=19$) was 31.2, 138 and 170 nmol/mL of plasma, respectively. The results indicate that the level of PC-Acro in plasma is well correlated with the severity of chronic renal failure. Furthermore, the level of PC-Acro returned toward normal after patients with chronic renal failure ($n=7$) had undergone hemodialysis [56]. Creatinine, used as a marker of chronic renal failure, is non-toxic. In some cases, creatinine level is high in the patients with moderate severity. Since acrolein is toxic and reflects cell damage, it may be useful if PC-Acro is measured as one of the markers of chronic renal failure along with creatinine. Accordingly, if subjects have both SBI and chronic renal failure, it is necessary to evaluate which is the major reason to increase PC-Acro by measuring another biochemical markers as well as imaging.

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disorder mainly affecting the salivary and lacrimal secretion caused by destruction of the glands. We measured PC-Acro in the saliva of pSS patients ($n=10$) and control subjects ($n=13$). Because the major protein conjugated with acrolein in the plasma was albumin [13], the level of albumin-Acro in the saliva was measured by Western blotting. The level of albumin-Acro in pSS patients was more than fivefold higher than that in control subjects [52]. The results also indicate that albumin-Acro is well correlated with the severity of pSS as assessed by the decrease in the flow rate of saliva.

7 Concluding remarks

Our results, altogether, indicate that acrolein is more toxic than ROS, and is produced primarily from SPM by sper-

mine oxidase. As there are no effective biochemical markers for the early period of brain infarction, it is important to develop effective biochemical markers because measurement of biochemical markers is more economical and easier compared with MRI and/or CT. Measurement of PC-Acro, IL-6 and CRP along with age makes it possible to detect SBI with 84% sensitivity and specificity. Biochemical markers also have an advantage to reflect the tissue damage at the day of blood collection compared with diagnostic imaging such as MRI and CT. Thus, both measurement of biochemical markers and diagnostic imaging to estimate the size of infarction are important for timely and appropriate therapeutic approaches.

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The authors have declared no conflict of interest.

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