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Time dependent transition of the levels of protein-conjugated acrolein (PC-Acro), IL-6 and CRP in plasma during stroke

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ABSTRACT

Objective: Measurement of plasma levels of protein-conjugated acrolein (PC-Acro) together with IL-6 and CRP can be used to identify silent brain infarction (SBI) with high sensitivity and specificity. The aim of this study was to determine how these biomarkers vary during stroke.
Methods: Levels of PC-Acro, IL-6 and CRP in plasma were measured on day 0, 2, 7 and 14 after the onset of ischemic or hemorrhagic stroke.
Results: After the onset of stroke, the level of PC-Acro in plasma was elevated corresponding to the size of stroke. It returned to near control levels by day 2, and remained similar through day 14. The degree of the decrease in PC-Acro on day 2 was greater when the size of brain infarction or hemorrhage was larger. An increase in IL-6 and CRP occurred after the increase in PC-Acro, and it was well correlated with the size of the injury following infarction or hemorrhage. The results suggest that acrolein becomes a trigger for the production of IL-6 and CRP, as previously observed in a mouse model of stroke and in cell culture systems. The increase in IL-6 and CRP was also correlated with poor outcome judging from mRS.
Conclusion: The results indicate that the degree of the decrease in PC-Acro and the increase in IL-6 and CRP from day 0 to day 2 was correlated with the size of brain infarction, and the increase in IL-6 and CRP with poor outcome at discharge.

1. Introduction

Polyamines (putrescine, spermidine and spermine) are synthesized from ornithine and *S*-adenosylmethione at the order of mM in cells, and mainly exist as a polyamine-RNA complex, where they stimulate several kinds of protein synthesis that are important for cell growth [1,2]. However, when cells are damaged, polyamines are released from RNA and the toxic compound acrolein (CH_2═CH—CHO) is produced by polyamine oxidases (spermine oxidase and acetylpolyamine oxidase) [3,4]. We examined whether the levels of polyamine oxidases and protein-conjugated acrolein (PC-Acro) in plasma are correlated with pathologies that involve tissue damage, and found that the levels of polyamine oxidases and PC-Acro in plasma are well correlated with the severity of chronic renal failure [5] and stroke [6]. It was also found that the induction of brain infarction in mice was correlated with increases in PC-Acro at the locus of infarction and in plasma [7].

It is thought that the major factor responsible for cell damage is reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH) [8]. However, when the toxicity of acrolein and ROS was compared, acrolein was more toxic than H_2O_2[9] and slightly more toxic than hydroxyl radical [10]. This finding supports an idea that PC-Acro becomes a biomarker for diseases accompanied with tissue or cell damage like renal failure [5], stroke [6], and Alzheimer's disease [11], because acrolein interacts efficiently with cysteine, lysine and histidine residues of proteins resulting in the inactivation of proteins.

There are reports that silent brain infarction (SBI) increases the risk of subsequent stroke [12–14], dementia [14] and mild cognitive

Abbreviations: PC-Acro, protein-conjugated acrolein; IL-6, interleukin-6; CRP, C-reactive protein; NIHSS, NIH stroke scale; mRS, modified Rankin Scale; SBI, silent brain infarction; WMH, white matter hyperintensity.

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impairment [15]. It has been also reported that carotid atherosclerosis (CA) is a risk factor for stroke and SBI [16,17] and that SBI and marked white matter hyperintensity (WMH) are risk factors for stroke [18]. We looked for biomarkers to estimate SBI and marked WMH, and found that measurement of PC-Acro together with interleukin-6 (IL-6) and C-reactive protein (CRP) makes it possible to identify SBI and marked WMH with high sensitivity and specificity [19]. Therefore, we studied how these three markers change during stroke. An increase in all three markers was seen after the onset of stroke in the order PC-Acro > IL-6 > CRP as observed in thrombosis model mice [20], and these three biomarkers were correlated with the size of stroke, and IL-6 and CRP with the outcome at discharge.

2. Materials and methods

2.1. Patients

Plasma samples were collected from 44 patients with brain infarction (28 males, 16 females; 73.5 ± 8.5 years of age), and 35 patients with intracerebral hemorrhage (21 males, 14 females; 65.0 ± 8.0 years of age), who were admitted to the Atsugi Municipal Hospital within 24 h after the onset of stroke.Stroke patients were defined as having focal infarcts or hemorrhage detected by magnetic resonance imaging (MRI) or computed tomography (CT) and managed according to the Japanese Guideline for the Management of Stroke (2009) [21]. In brief, patients were treated with anticoagulants and/or antiplatelets with or without edaravone, a free radical scavenger for 7 to 14 days, and dextran for 5 days after the stroke. None of the patients received immunodepressive medicines. Blood was collected from patients 4 times (day 0 on admission, day 2, 7 and 14) using procedures approved by the ethics committees of Atsugi Municipal Hospital and Graduate School of Pharmaceutical Sciences, Chiba University.Informed consent was given by patients or by their relatives as legally required.Clinical investigations were conducted in accordance with the Declaration of Helsinki principles.Blood containing 3 U/ml heparin was centrifuged at 1500 x g for 10 min at 4 °C. The plasma was carefully collected to avoid contamination by erythrocytes, and kept at −80 °C.

2.2. Measurement of PC-Acro, IL-6 and CRP

PC-Acro [N^{ε}-(3-formyl-3,4-dehydropiperidino)-lysine (FDP-lysine) in protein] was determined by the method of Uchida et al. [22] using ACR-LYSINE ADDUCT ELISA SYSTEM (NOF Corporation) and 0.01 ml plasma.IL-6 and CRP were measured using human IL-6 ELISA kit (R & D Systems) and N-assay LA CRP-S kit (Nittobo), respectively, according to the manufacturer's protocol.

2.3. Imaging, NIH stroke scale (NIHSS), modified Rankin Scale (mRS) and assessment of outcome

All patients underwent T1- and T2-weighted MRI, fluid-attenuated inversion recovery (FLAIR), diffusion weighted image (DWI), and CT. MRI was performed in 7-nm thickness with 1- to 2-nm slice gap with a 1.5-T MRI unit (Signa: GE Medical Systems). A standard head coil with a receive-transmit birdcage design was used. The maximum size of focal infarcts were measured using 5 or 10 nm length calibration accompanied in each image [6]. The volume of intracerebral hemorrhage was measured using the CT image according to the method of Broderick et al. [23]. NIHSS was evaluated on admission according to the method of Brott et al. [24]. Modified RS (score 0–6, 0, no symptoms at all; 1, no significant disability despite symptoms; 2, slight disability; 3, moderate disability; 4, moderately severe disability; 5, severe disability; 6, dead) was assessed according to the method described by Shinohara et al. [25] at the day of discharge. Assessment of the outcome was performed according to Glassgow Outcome Scale: Good outcome, good recovery; Poor outcome, moderately disabled, severely disabled and persistent vegetative state [26].

2.4. Statistics

Statistical calculations were performed with GraphPad Prism® Software (GraphPad Software). Values are indicated as median ± interquartile deviation or median with interquartile range. Groups were compared using Wilcoxon rank sum test, Wilcoxon signed-rank test, Kruskal-Wallis test, chi-test or Fisher's exact test. Correlations between each factor were examined by Spearman's rank correlation analysis to obtain the correlation coefficient (r_s) and p value. False Discovery Rate (FDR) correction [27] was used for multiple comparison.

3. Results

3.1. Time course of the levels of PC-Acro, IL-6 and CRP during brain infarction and hemorrhage

As shown in Table 1, the number of brain infarction and hemorrhage patients was 44 (73.5 ± 8.5 years of age) and 35 (65.0 ± 8.0 years old), respectively, at the onset of the study. Three biomarkers (PC-Acro, IL-6 and CRP) were followed for 15 days (days 0, 2, 7 and 14) after the onset of the stroke (Fig. 1). The level of PC-Acro on day 0 was higher in both brain infarction and hemorrhage. However, unexpectedly, the level of PC-Acro in brain infarction patients was reduced on day 2 compared to day 0, and the level remained similar through day 14. Although the level of PC-Acro in patients with brain hemorrhage was lower on day 0 than that in patients with brain infarction, a similar reduction on day 2 compared to day 0 was observed. In both groups of patients, the level of IL-6 on day 2 increased about 2-fold compared to that on day 0, and gradually decreased from day 2 to day 14 (Fig. 1A). The level of CRP was maximal on day 7 and subsequently decreased by day 14 in both groups of patients (Fig. 1A). The results suggest that production of PC-Acro at the locus of brain stroke may be a trigger for the production of IL-6 and CRP, as has been observed in a mouse model of stroke and in cell culture systems [20]. PC-Acro was higher in brain infarction, and the IL-6/CRP ratio was higher in brain hemorrhage on day 0, suggesting that the orderly increase in PC-Acro, IL-6 and CRP may be rapid in brain infarction than hemorrhage. The results strongly suggest that three biomarkers, measured early after a stroke, may be useful biomarkers to differentiate brain infarction from brain hemorrhage.

Similar results were obtained with age-matched patients of brain infarction (37 patients, 72.0 ± 6.0 years old) and hemorrhage (35 patients, 65.0 ± 8.0 years old) (data not shown).

3.2. Dependency of the increase in three biomarkers on the size of brain infarction and hemorrhage

It was then determined whether levels of the biomarkers were correlated with the size of brain damage following infarction and hemorrhage. For this analysis, patients with brain infarction and hemorrhage were classified into two groups in which numbers were nearly equal in small and large groups. As shown in Fig. 2AB, the degree of the decrease in PC-Acro from day 0 to day 2 and that of the increase in IL-6 and CRP from day 0 to day 2 or 7 were correlated with the size of brain infarction and hemorrhage. These results indicate that measurements of PC-Acro together with IL-6 and CRP at the early period of brain stroke contribute to the judgement of the infarct size and hemorrhage volume.

Since the size of cardioembolic (CE) infarction was bigger than that of atheroembolic including lacnar (non-CE) infarction (Supplementary Table 1S), it was determined whether there were differences in the three biomarkers in patients with CE and non-CE brain infarctions (i.e., subsets of the “infarction” group presented in Supplementary