의학 석사학위 논문

The Use of Ischemia-modified Albumin (IMA) in Ischemic Stroke

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안 정 환

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안정환의 의학 석사학위 논문을 인준함.

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- ABSTRACT -

The Use of Ischemia-Modified Albumin (IMA) in Ischemic Stroke

Introduction : The aim of this study was to investigate the possibility of ischemia-modified albumin (IMA) as a new and rapid diagnostic biomarker of ischemic stroke and compare IMA in progression of stroke and non-progression of ischemic stroke.

Methods : Fifty-two emergency patients with acute neurologic symptoms were admitted to the emergency department within 6 hours of symptom onset (28 Ischemic Stroke Group; SG, 24 Non-Stroke Group; NSG). Blood samples for IMA measurements by albumin cobalt binding test (Ischemia Technology, Denver, U.S.A) were obtained at arrival, and 12 and 24 hours after the onset of symptoms.

Results : The initial mean IMA level was 107.4 (107.4±11.0) U/ml in SG and 86.5 (86.5±8.5) U/ml in NSG, respectively (p <0.001). Fifteen SG patients showed progression. The difference between the two groups in IMA level according to time was not statistically significant (p > 0.05).

Conclusion : IMA may be a new diagnostic biomarker in ischemic stroke. However, it is difficult to be used as a predictive biomarker for progression of ischemic stroke.

Key words : Ischemia-modified albumin, Ischemia, Ischemic stroke

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Ⅰ. INTRODUCTION

The treatment of ischemic stroke within 3 hours after symptom onset with thrombolytic agents or percutaneous transluminal recanalization can avoid neurological damage. Therefore, it is a disease requiring rapid diagnostic approach and early treatment determines the prognosis (Davis et al., 2005). Thus an accurate and rapid diagnostic method needs to be developed for ischemic stroke.

For the accurate and rapid diagnosis of ischemic stroke, we focused on ischemia-modified albumin (IMA). IMA is a modified protein which has a decreased ability to bind to cobalt, and which is generated under the ischemic condition (Sadler et al., 1994; Bar-Or et al., 2000). IMA that may have a “rule-out” role in selected patients with acute coronary syndrome through a negative result in the triple predictive test (Bar-Or et al., 2000 ; Peacock et al., 2006).

In the previous study, the IMA level according to time in the stroke group was different from the aspect of myocardial ischemia (Abboud et al., 2007).

In this study, the potential for IMA to act as a marker in the rapid detection of ischemic stroke was evaluated based on the characteristics.In addition, to investigate the characteristics of IMA, we confined ischemic tissue to ischemic stroke and tried to analyze IMA level according to the time between progressive and non-progressive ischemic stroke. The progression group was defined when National Institutes of Health Stroke Scale was increased.

Ⅱ. METHODS

A. Patent enrollment

This study was conducted prospectively for 3 months from September 1, 2006 in an emergency medical center of a university affiliated hospital. The study subjects were those patients admitted with neurological symptoms as the chief complaint and who underwent neurological test, brain computed tomography (CT), and brain magnetic resonance imaging (MRI). The patients diagnosed as stroke in brain MRI were classified in the brain ischemic stroke group (SG) and those without special findings in the non-stroke group (NSG). In SG, the progression and non-progression groups were classified. The progression group was defined when National Institutes of Health Stroke Scale was increased.

Cases which passed 6 hours from symptom onset, those with a past history of brain hemorrhage, and those with myocardial ischemia, cardiac arrest, renal failure, and major, multiple surgeries were excluded, as were those who did not give consent for the IMA test.

This study was conducted after obtaining the approval of the review of the Institutional Review Board of Ajou University Hospital (Suwon, South Korea)

B. Data collection

In SG, IMA was measured at the time of admission, and at 12 and 24 hours after symptom onset. In NSG, patients were discharged within 12 hours of symptom onset, so IMA and albumin values were measured only at the time of admission.

Serum samples for IMA were immediately centrifuged, and those samples were

stored at -20℃ for later analysis. IMA was measured by albumin cobalt binding (ACB) test (Ischemia Technology, Denver, U.S.A) on a TBA-200FR analyzer (Toshiba Medical Systems Co., Ltd., Tokyo, Japan). Within-assay (n=10) imprecision, as expressed by the coefficient of variation, was ≤ 2%.

C. Statistical analysis

52 patients who had suddenly developed neurological symptoms during the study period were finally selected. Data and receiver operating characteristic (ROC) curve were analyzed using the SPSS 12 statistics program(SPSS Inc., USA) and General+ Clinical laboratorystatistics version 1.73 (Analyse-it software, Ltd., England United Kingdom). For the general validation of statistics between the two groups and IMA comparison, Mann-Whitney U test and Chi-square test were performed. The cases with p value less than 0.05 were determined to be statistically significant. We constructed a ROC curve to identify the optimal threshold by maximizing the sum of sensitivity and specificity. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for the optimum cutoff point of 80 U/ml recommended in the previous pilot study and manufacturer cutoff point of 85 U/ml. In SG, two-sample Kolmogorov-Smirnov Z was performed to compare the brain stroke patients with and without progression.

Ⅲ. RESULTS

A. General characteristics of the subject group

The final subjects were 52 cases: 28 SG (53.8 %) and 24 NSG. The mean age of the sample was 60.2±16.3 years, and the male/female ratio was 1 : 1.4: 59.3±16.5 years and 1 : 1.3 in SGand 61.3±16.2 years and 1 : 1.4 in NSG, respectively. There was no significant difference in mean age and gender ratio between the groups (p=0.66, p=0.87).

B. IMA value at the time of admission of each group

At the time of admission, the mean IMA value was 107.4 (107.4±11.0) U/ml and 86.5 (86.5±8.5) U/ml in SG and NSG, respectively. IMA at the time of admission was significantly different between the two groups (p<0.001). (Table 1, Fig 1)

The ROC curve is shown in Fig 2. The area under the curve is 0.928 (95% CI, 0.857-0.999). A cutoff value of 98 U/ml appeared to be optimal, giving sensitivity 87.5 %, specificity 89.3 %, PPV 87.5 % and NPV 89.3 %. Using the recommended manufacturer cutoff of 85 U/ml, the sensitivity of IMA for the diagnosis of stroke decreased to 45.8 %, with a specificity of 96.4 %, PPV 91.7 % and NPV 67.5 %. Using the recommended previous pilot study cutoff of 80 U/ml, the sensitivity of IMA for the diagnosis of stroke decreased to 25 %, with a specificity of 100 %, PPV 100% and NPV 60.9 %.

Table 1. IMA* level of each group

	Patients		*p*-value (t value)
	Stroke group	**Non-Stroke group**	
IMA* (U/ml)	107.4±11.0	86.5±8.5	***P*<0.001 (8.8)**

* IMA: Ischemia modified albumin

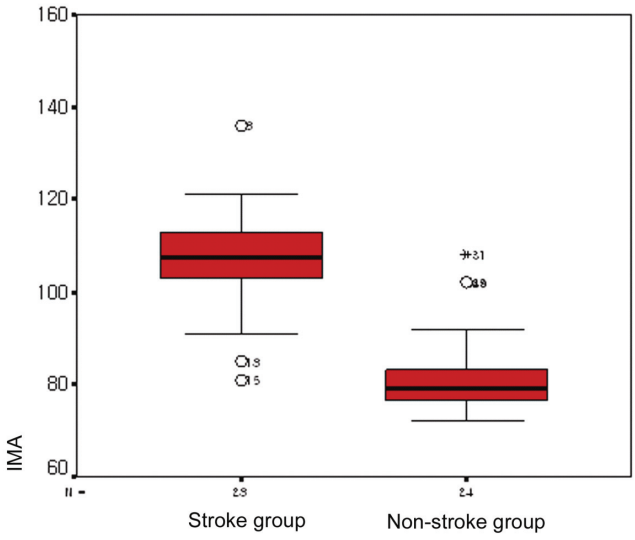

Fig. 1. Average IMA* level of each group. At the time of admission, the mean IMA value was 107.4 (107.4±11.0) U/ml and 86.5 (86.5±8.5) U/ml in SG and NSG. IMA; Ischemia-modified albumin.

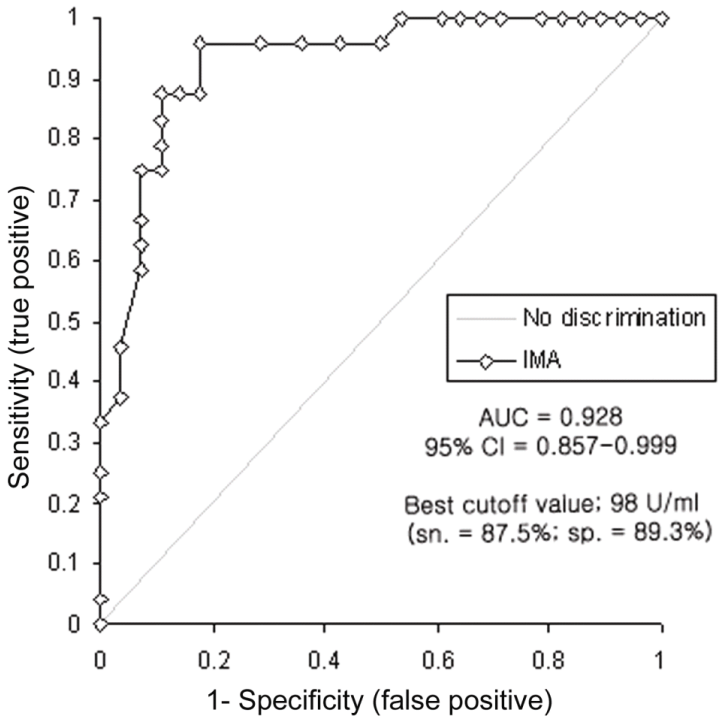

Fig. 2. Receiver operating characteristic curves of initial IMA for diagnosis of acute ischemic stroke. A cutoff value of 98 U/ml appeared to be optimal, giving sensitivity 87.5 %, specificity 89.3 %, PPV 87.5 % and NPV 89.3 %. AUC = Area under the curve ; sn. = sensitivity ; sp. = specificity.

C. IMA value and comparison according to time points in the brain stroke group

IMA value at the time of admission, and at 12 and 24 hours after symptom onset was 107.4 (107.4±11.0) U/ml, 105.2 (105.2±13.9) U/ml, and 109.5 (109.5±10.8) U/ml, respectively. IMA value according to each time point between progression and non-progression SG was not significantly different. (Table 2)

Table 2. The change of IMA* level according to time in the Stroke Group.

	Initial IMA*(U/ml)	12hr IMA*(U/ml)	24hr IMA*(U/ml)
Progression	108.5±11.9	105.8±12.4	110.3±12.4
Non-Progression	106.1±10.0	104.6±16.0	108.8±8.7
Z score	0.49	0.83	0.62
***p*-value**	0.97	0.50	0.83

* IMA: Ischemia modified albumin

3.4. The IMA value of the group using thrombolytic agents or percutaneous transluminal recanalization

IMA value of 3 patients treated by thrombolytic agents was lowered after use of thrombolytic agents but increased again as the brain stroke progressed. In one patient treated by percutaneous transluminal recanalization, IMA value measured 12 hours later was in the low to normal range without special complications. The change of IMA value measured subsequently is shown in Table 3.

Table 3. The change of IMA* level in stroke patients treated by thrombolytics or intervention

Patient	Initial IMA*(U/ml)	12hr IMA*(U/ml)	24hr IMA*(U/ml)
a	117	87	87
b	109	114	113
c	121	117	118
d	103	85	97

* IMA: Ischemia modified albumin

Patient a : The patient was stable. The intra-arterial stent insertion was done.

Patient b: Progressive symptoms appeared at 4hours after the onset of initial symptoms. At that time, thrombolytics were used. After using thrombolytics, the patient was stable.

Patient c: Thrombolytics were used at 2hours after the onset of initial symptoms. Progressive symptoms appeared at 15hours after the onset of initial symptoms.

Patient d: Thrombolytics were used at 6hours after the onset of initial symptoms. Progressive symptoms appeared at 18hours after the onset of initial symptoms.

Ⅳ. DISCUSSION

Albumin is a protein consisting of 585 amino acids, with a molecular weight of 66,500 daltons and a characteristic terminus in each organism (Sadler et al., 1994). Exposure to certain conditions such as ischemia, reperfusion and oxidation, whereby free radicals are generated, modifies circulating albumin at its NH_2 terminus by different mechanisms (Christenson et al., 2001; Apple et al., 2002; Garrido et al., 2004; Sinha et al., 2004; Zapico-Muniz et al., 2004; Peacock et al., 2006; Abboud et al., 2007).

Several studies revealed that free radical production increases in stroke, particularly with ischemia and reperfusion (Zini et al., 1992). This fact reveals that IMA can be generated in ischemic stroke. A recent pilot report showed significant IMA increases after stroke and IMA level on admission correlates with National Institutes of health Stroke Scale (NIHSS) (Abboud et al., 2007). According to our study results, although the number of cases was small which limited the statistical power of the conclusions, IMA in SG was significantly increased in comparison with NSG. This supports the value of IMA as a good biological marker to diagnose brain stroke relatively rapidly, although additional studies will be required for confirmation.

However, the mean value of IMA and the cutoff value in this study were higher than the previous study (Abboud et al., 2007). In case of the mean value, the previous study on the usefulness of IMA in stroke suggests 83 U/ml (79-86 U/ml). However, in our study, the mean value of IMA was higher than the previous study at 107.4 U/ml and in NSG was at 86.5 U/ml which had even higher result. The cutoff value was also higher than the suggested value for the myocardial ischemia by the manufacturer as well as the previous study. Using the cutoff value recommended by manufacturer and previous study, sensitivity, specificity and NPV had little merit for the diagnosis of ischemic stroke. Additionally, 70.8 % of NSG had higher results

than the cutoff value 80 U/ml. It has been reported that the mean value of IMA of a healthy Asian was 91.2±5.4 U/ml (range: 77~105 U/ml) which was a higher reference value than non-Asians (Kim et al., 2005). Although a reference value has not been defined for Asians, there is an assumption that Asians will show higher reference value due to the difference in ethnic background or diet. According to Abadie (Abadie et al., 2005)et al., the results from each institute and equipment vary. Thereupon, it was recommended that the cutoff value be analyzed through an ideal ROC curve in each examination room. Even though this is a preliminary report, we suggest that IMA analysis for each ethnic background should be attempted and unification of test equipments for IMA value must be promoted.

In SG, IMA values according to each time point were not significantly different. In myocardial ischemia, IMA was increased within a few minutes, continued to increase up to 6 hours, and subsequently disappeared after 12 hours (Garrido et al., 2004). However, IMA characteristics depending on time were not shown as in myocardial ischemia based on this study. Abboud H (Abboud et al., 2007)et al. explained the possibility of an increase in oxidative stress due to oligemia in the ischemic penumbra and that reperfusion might result in an increase in IMA concentrations within the first 24 h of onset in ischemic stroke. The change of IMA value depending on time, shown in table 2, did not show any significant difference between the progression and non-progression groups. IMA should not be used as a biomarker for the progression of ischemic stroke for the time being, for the reason detailed above.

An interesting fact discovered from this study is that the IMA value of patients with reperfusion had decreased after reperfusion therapy. Regarding the use of thrombolytic agents in 3 cases and the performance of percutaneous transluminal recanalization in one case in SG, the limited number of cases prevents any generalized conclusions being made. Nevertheless, the four cases suggest that IMA could be decreased after reperfusion therapy, similar to ischemic cardiac disease. In

brain stroke patients, a few IMA measurements prior to and after reperfusion therapy might be useful to determine the effect of reperfusion therapy. However, additional studies will be required to confirm this.

The limitations of our study were that first, the number of cases was small which limited the statistical power of the conclusions. Second, the study was unable to state the differences in IMA characteristics on various ethnic backgrounds because it was held in a homogeneous country. Third, it may not be a genuine NSG because transient ischemic attack may be included. However, according to Abboud H (Abboud et al., 2007) et al., IMA value in transient ischemic attack was lower than in stroke. Additional study in transient ischemic attack will be necessary since the previous study was a pilot study. Last, the range of the brain ischemic stroke group was too broad. In future studies, the IMA characteristics should be further examined by classifying the subdivided study groups such as pathophysiology, location of cerebral infarction and so on.

We suggest that IMA studies should be conducted for various ethnic groups. Moreover, measuring IMA prior to and after the use of thrombolytic agents and percutaneous transluminal revascularization will elucidate the IMA characteristics and effectiveness of reperfusion therapy.

Ⅴ. CONCLUSION

IMA was increased in ischemic stroke patients. Therefore, IMA may be a new diagnostic biomarker in ischemic stroke. IMA should not be used as a biomarker for the progression of ischemic stroke for the time being.

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– 국문 요약 –

허혈성 뇌혈관 질환에서 Ischemia-modified albumin의 유용성

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(지도교수 : 이 국 종)

목적: 뇌경색이나 허혈성 뇌질환은 빠르고 정확한 진단방법을 필요로 한다. 조직이 허혈상태에 있을 경우 Ischemia-modified albumin(IMA)이 생성된다. 본 연구의 목적은 IMA가 뇌경색이나 뇌허혈성 질환이 있을 때의 변화를 관찰하여 허혈성 뇌질환을 초기에 빠르게 진단할 수 있는 생리학적 지표로써의 가능성을 알아보고자 하였다.

대상 및 방법: 6시간 이내에 발생한 신경학적 증상을 주소로 내원한 52명을 대상으로 하였다. 모든 대상들은 두경부 전산화 촬영과 두경부 자기 공명 촬영을 시행하였다. 영상 검사에서 뇌경색의 증거가 있는 경우가 28명이었고 이 군을 뇌경색군이라 명명하였고 영상 검사에서 뇌경색의 증거가 없는 경우는 24명으로 이 군을 비뇌경색군이라 명명하였다. 내원시, 증상발현 12시간 후, 증상발현 24시간 후에 각 환자에서 IMA를 측정하였다. 통계학적 검증은 통계 프로그램인 SPSS 12.0 (SPSS Inc., USA)과General+Clinical laboratory statistics version 1.73(Analyse-it software, Ltd., England United Kingdom)를 이용하였다. Receiver operating characteristic (ROC)

curve를 이용하여 판정 기준치(cutoff value)를 산출하였고, Mann-Whitney U test, Chi-square test를 이용하여 뇌경색군과 비뇌경색군의 IMA 수치를 비교하였다. 진행성 뇌경색군과 비진행성 뇌경색군을 비교하기 위하여 two-sample Kolmogorov-Smirnov Z를 이용하였다.

결과: 뇌경색군과 비뇌경색군의 평균 연령은 각각 59.3(59.3±16.5), 61.3(61.3±16.2)이었다. 통계학상 의미가 없었다 ($p>0.05$). 뇌경색군과 비뇌경색군의 내원시 IMA 수치는 107.4(107.4±10.68) U/ml, 82.0(82.0±9.86) U/ml이었다. 각군의 IMA 수치는 통계학적으로 유의하였다 ($p<0.001$). 뇌경색군의 내원 당시, 증상 발현 12시간, 24시간 뒤의 IMA 수치는 각각 107.4(107.4± 10.68)U/ml, 105.3(105.3±13.9) U/ml, 109.5(109.5±10.8)U/ml)이었다. 가장 적절한 판정기준치는 98 U/ml이었다. 뇌경색군에서 각 시간대에 따른 IMA 수치는 뇌경색의 진행 유무에 관계없이 통계학으로 유의하지 않았다 ($p>0.05$). 본 연구에서 혈전 용해제 혹은 관혈적 재개통술을 시행한 환자는 총 4명이었고 투약 혹은 시술 후에 IMA 수치는 떨어지는 양상을 보였다.

결론: 본 연구를 통하여 뇌경색 환자는 IMA가 증가하며, 뇌경색이 진행되는 상황에서도 IMA는 증가하는 것으로 보인다. 따라서 IMA는 허혈성 뇌경색에서 빠르게 진단할 수 있는 생화학적 지표로 사용될 수 있을 것이다.

핵심어: Ischemia-modified albumin, 허혈, 허혈성 뇌질환