Plasma Endothelin-1 Levels in Patients with Rheumatic Mitral Stenosis and A History of Cerebral Thromboembolism

Li-Chuan Ma, RN; Mien-Cheng Chen, MD; Hsueh-Wen Chang, PhD[1]

Background: Increased plasma endothelin-1 concentrations have been observed in patients with rheumatic mitral stenosis. Endothelin-1 levels have never been investigated in patients with mitral stenosis and history of cerebral thromboembolism.

Methods: We measured plasma concentrations of endothelin-1 in the peripheral venous blood samples obtained from 20 patients with moderate to severe rheumatic mitral stenosis (16 with permanent atrial fibrillation and 4 with sinus rhythm). Six patients had history of thromboembolism. The remaining 14 patients did not have history of thromboembolism. Plasma endothelin-1 concentrations were measured using solid phase sandwich enzyme linked-immuno-sorbent assay.

Results: The peripheral venous concentrations of endothelin-1 of the six patients with history of thromboembolism did not differ from the concentrations of the 14 patients without history of thromboembolism (2.40 ± 1.39 pg/ml vs. 2.49 ± 0.66 pg/ml, $p = 0.9$).

Conclusions: Although plasma endothelin-1 concentrations were increased in patients with mitral stenosis, plasma endothelin-1 concentrations were not further elevated in patients with mitral stenosis and history of thromboembolism.
(Chang Gung Med J 2004;27:794-800)

Key words: mitral stenosis, endothelin-1, cerebral thromboembolism.

Endothelin-1 (ET-1) is an endothelium-derived vasoconstrictor peptide with 21-amino acid residues originally isolated from culture media conditioned by porcine aortic endothelial cells.[1] We and other researchers have demonstrated that circulating ET-1 is elevated in patients with mitral stenosis (MS) compared with healthy subjects.[2-6] Several studies of stroke patients have shown elevated levels of plasma ET-1 in patients with various subtypes and phases of brain infarction.[7-10] However, contradictory results have also been presented.[11,12] Anwaar and associates reported that at the acute stage of cerebral ischemia,[13] there were no differences in plasma ET-1 levels among patients with large-vessel disease, small-vessel disease and cardioembolic disease. However, after 1 year of follow-up, plasma ET-1 levels were higher in patients with cardioembolic disease when compared with patients with small- and large-vessel disease.[13] Plasma ET-1 levels have never been investigated in patients with MS and history of cerebral thromboembolism. Accordingly, we tested the hypothesis that in patients with MS, the

From the Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, Kaohsiung; [1]Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung.
Received: Feb. 10, 2004; Accepted: Sep. 7, 2004
Address for reprints: Dr. Mien-Cheng Chen, Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital. No. 123, Dabi Rd., Niausung Shiang, Kaohsiung, Taiwan 833, R.O.C. Tel.: 886-7-731-7123 ext. 2363; Fax: 886-7-732-2402; E-mail: chenmien@ms76.hinet.net

plasma ET-1 levels were increased in patients with history of cerebral thromboembolism.

METHODS

Study population

Twenty patients who had symptomatic rheumatic MS without significant mitral, tricuspid or aortic regurgitation and left atrial thrombus and had undergone percutaneous transluminal mitral valvuloplasty were studied. There were two men and 18 women ranging in age from 39 to 72 years (mean, 55±12 years). Sixteen patients had permanent atrial fibrillation and four had sinus rhythm. Six patients had history of cerebral thromboembolism, which occurred more than 6 months before valvuloplasty (group 1). The diagnosis of cerebral thromboembolism was confirmed using the results of complete neurologic examinations that included brain computer tomographic scans. None of the six patients had cerebral ischemia due to atherothrombosis, subarachnoid hemorrhage, intracerebral hemorrhage, hematoma, or complicated migraine and none of them had arterial stenosis or occlusion as a potential source of embolism. The remaining 14 patients constituted group 2. Ten patients were in the New York Heart Association (NYHA) functional class III and 10 were in the NYHA functional class II. In group 1, two patients had sinus rhythm and four had permanent atrial fibrillation which was defined as persistent atrial fibrillation lasting > 4 weeks. In group 2, two patients had sinus rhythm and 12 had permanent atrial fibrillation. No patient had a history of malignancy, inflammatory disease, collage vascular disease, renal or liver disease, diabetes mellitus, hypertension, hyperlipidemia, infectious disease, deep venous thrombosis, pulmonary embolism or recent surgery. Peripheral venous plasma ET-1 concentrations were measured in all of the patients studied. The Institutional Review Committee on Human Research at our institution approved the study protocol.

Doppler echocardiography and medications

Transthoracic echocardiographic examinations were performed on the day of valvuloplasty and before the valvuloplasty procedure with a 2.5 MHz transducer attached to a commercially available echo Doppler machine (Sonos 5500; Hewlett-Packard; Palo Alto, Calif) to assess left atrial dimension and mitral valve area. M-mode measurements were performed according to the recommendation of the American Society of Echocardiography. The mitral valve area was calculated using the Doppler pressure half-time method. Left and right atrial areas were planimetered in the four-chamber view, and the maximum areas were measured (at the end of the T wave on the electrocardiogram) and averaged over 5 beats. The severity of mitral, tricuspid and aortic insufficiency was determined using Doppler color-flow mapping. The absence of left atrial cavity or appendageal thrombus was confirmed using transesophageal echocardiography.

Warfarin was discontinued for at least 3 days before the study began. Diuretics were discontinued on the day the study began. Digoxin and Ca-blockade were discontinued for at least five half-life before the study began.

Hemodynamic measurements

The mean pressures in the right atrium, left atrium, and ascending aorta, and the transmitral pressure gradient were obtained before valvuloplasty.

Blood sample collection and measurement of plasma ET-1 concentrations

Blood samples were obtained during the fasting, non-sedative state at 9-10 AM to exclude the possible influence of circadian variations and from the femoral vein through introducer sheaths immediately after puncture with the patients in the supine position for at least 20 minutes.[8,14,15] Five mL of blood was drawn into an evacuated tube containing K_3 ethylenediamine tetra acetic acid (Vacutainer; Becton Dickinson; Franklin, Lakes, NJ). Blood samples with gross hemolysis were discarded. Mixtures of blood and K_3 ethylenediamine tetra acetic acid were immediately centrifuged (model 5400; Kubota Corp; Tokyo, Japan) at 3000 revolutions per minutes for 10 minutes at room temperature. The plasma was immediately separated and frozen at -80˚C until the assay. Blood samples were also withdrawn for whole blood counts, biochemical and electrolytes measurements using standard laboratory methods.

The ET-1 concentrations of human plasma samples were quantified using a commercially available solid phase sandwich enzyme linked-immuno-sorbent assay kit (R and D Systems; Minneapolis, Minn) after extraction. The samples were processed

according to the manufacturer's instructions. Because the solid phase sandwich enzyme linked-immuno-sorbent assay consist of two antibodies, they had higher specificity than conventional radioimmunoassays.[16] Before the assay, 500 μL plasma samples were thoroughly mixed with 750 μL of extraction solvent [acetone: 1N HCL: water (40:1:5)] and centrifuged at 14,000 revolutions per minutes for 20 minutes in a refrigerated centrifuge at 4˚C. The supernatant was decanted and dried under reduced pressure in a centrifugal evaporator (Speed Vac SC110, Refrigerated Vapor Trap RVT100, Valupump VLP120; Savant Instruments Inc.; Holbrook, NY). The pellet was reconstituted in 0.25 mL sample diluent and vortexed for 30 seconds. The samples, which included standards in buffer and reconstituted extracts of the quality control and test samples and an enzyme (horseradish peroxidase)-labeled second antibody, were sequentially added to a 96-well microplate precoated with an anti-ET-1 antibody. After 1 hour of incubation at room temperature and removal of unbound materials, the amount of enzyme-conjugated tracer bound to the wells was detected through the reaction with a substrate specific for the enzyme. The reaction product was measured using a microplate reader (MRX; Dynex Technologies, Inc.; Chantilly, Va) and reading the absorbance at 450 nm with a correction wavelength of 630 nm. The assay was sensitive to detect less than 1.0 pg/mL of ET-1. The cross-reactivity of ET-2, ET-3 and big ET in this assay were 45%, 14% and <1%, respectively, according to the manufacturer of the assay kits. A standard curve was determined with the use of the mean absorbance values of the included ET-1 standards, and the ET-1 concentrations in all unknown plasma samples were then calculated with linear regression. All standards and samples were tested in duplicate. The mean intra-assay coefficient of variance was 6.2% in our laboratory.

Statistical analysis

Continuous variables were described as mean ± SD. Categorical variables were compared using the Fisher's exact test (2-tail). Continuous variables between the two groups were compared using the Student t test (2-tail). Statistical analysis was performed using the computer software program SAS for Windows version 8.2 (SAS Institute, Cary, NC). A *p*-value of 0.05 or less was considered statistically significant.

RESULTS

Comparison of baseline characteristics and peripheral venous plasma ET-1 levels between the two groups

The baseline characteristics for each group are summarized in Table 1. There were no significant differences between the two groups in terms of age, gender, percentage of patients in atrial fibrillation, the use of digoxin, Ca-blockade, amiodarone, propafenone and warfarin, blood cell counts and biochemistry data. The mean duration of atrial fibrillation among the group 1 patients with atrial fibrillation was significantly longer than that of the group 2 patients with atrial fibrillation. The venous plasma ET-1 levels among the group 1 patients did not differ from those of the group 2 patients.

Table 1. Baseline Characteristics and Peripheral Venous Plasma ET-1 Concentrations of Patients Studied

Variables	Group 1 (n = 6)	Group 2 (n = 14)	*p*
Age (years)	55 ± 13	56 ± 12	0.881
Men (%)	16.7	7.1	0.521
% in AF rhythm	66.7	85.7	0.549
Duration of AF (months)	93.0 ± 45.5	25.8 ± 24.6	<0.002
Digitalis (%)	66.7	71.4	1.0
Warfarin (%)	100	92.9	1.0
Amiodarone (%)	0.0	7.1	1.0
Propafenone (%)	0.0	7.1	1.0
Ca-blockade (%)	16.7	28.6	1.0
Platelet ($\times 10^4$/uL)	20.2 ± 2.6	19.6 ± 5.7	0.803
Hemoglobin (gm/dL)	12.7 ± 1.0	12.1 ± 1.9	0.545
Leukocyte ($\times 10^3$/uL)	7.0 ± 3.3	6.2 ± 0.9	0.566
Calcium (mg/dL)	9.0 ± 0.4	8.7 ± 0.4	0.288
Creatinine (mg/dL)	1.02 ± 0.31	0.97 ± 0.32	0.774
ET-1 (pg/mL) in the PVB	2.404 ± 1.394	2.486 ± 0.656	0.896

Abbreviations: AF: atrial fibrillation; ET-1: endothelin-1; PVB: peripheral venous blood.
Data presented are mean ± SD or percentage of patients.

Comparison of hemodynamic variables and echocardiographic parameters between the two groups (Table 2)

There were no significant differences between the two groups in terms of the left atrial dimension, left ventricular dimension, left ventricular ejection fraction and mitral valve area. There were also no

Table 2. Hemodynamic Variables and Echocardiographic Parameters of Patients Studied

Variables	Group 1 (n = 6)	Group 2 (n = 14)	*p*
Arterial blood pressure (mmHg)	105.7 ±17.7	98.2 ±12.5	0.294
Right atrial pressure (mmHg)	8.3 ± 7.1	8.4 ± 5.7	0.994
Left atrial pressure (mmHg)	23.8 ± 6.9	22.6 ± 4.4	0.644
Transmitral pressure gradient (mmHg)	12.6 ± 5.7	10.8 ± 4.0	0.413
Left atrial diameter (mm)	46.2 ± 7.3	48.0 ± 6.9	0.599
Left atrial area (cm^2)	34.6 ± 7.1	37.2 ±12.6	0.648
Right atrial area (cm^2)	19.5 ± 5.1	22.0 ± 8.8	0.527
LVEDD (mm)	45.7 ± 6.3	46.1 ± 4.6	0.851
LVESD (mm)	28.7 ± 5.6	29.1 ± 3.6	0.847
LVEF (%)	66.5 ±10.6	66.4 ± 7.8	0.973
Mitral valve area (cm^2)	1.10 ± 0.19	1.04 ±0.17	0.514

Abbreviations: LVEDD: left ventricular end-diastolic diameter; LVEF: left ventricular ejection fraction; LVESD: left ventricular end-systolic diameter.
Data presented are mean ±SD.

significant differences between the two groups in terms of the left and right atrial pressures, aortic pressure, and transmitral pressure gradient.

DISCUSSION

In the present study, we examined the plasma ET-1 levels in peripheral venous blood samples of patients with symptomatic rheumatic MS, which produced two major findings. First, the peripheral venous plasma ET-1 levels among patients with history of cerebral thromboembolism did not differ from those of patients without history of cerebral thromboembolism. Second, the duration of atrial fibrillation among patients with atrial fibrillation and history of cerebral thromboembolism was significantly longer than that of patients with atrial fibrillation and without history of cerebral thromboembolism.

Plasma ET-1 levels among patients with history of cerebral thromboembolism

In the very early stages of nonhemorrhagic cerebral stroke (within 18 hours of stroke onset), ET-1 was found to be significantly elevated in the cerebrospinal fluid, while no elevation was demonstrated in the plasma.[11] However, plasma ET-1 tended to be elevated at 24 hours and decline thereafter after stroke onset.[7,9] Anwaar and associates[13] reported that after 1 year of follow-up after ischemic stroke, plasma ET-1 levels were higher in patients with cardioembolic disease when compared with patients with small- and large-vessel disease. However, only nine patients had cardioembolic disease in their study. Recently in a large clinical study, Haapaniemi et al demonstrated that plasma ET-1 levels were normal in patients with ischemic stroke during different time frames after stroke onset when compared with age- and sex-matched healthy control subjects.[12] Their findings were consistent with previous study that there were dissimilarities between the cerebrospinal fluid and plasma ET-1 concentrations in patients with ischemic stroke.[11] In addition, they also found that there was no correlation between plasma ET-1 levels and stroke etiology. In our study, we found that in patients with MS, the venous plasma ET-1 levels among patients with history of cerebral thromboembolism did not differ from those of patients without history of cerebral thromboembolism. ET-1 is released to the abluminal side of the vasculature;[17,18] consequently, plasma ET-1 levels probably do not accurately reflect the true magnitude of the increase in ET-1 levels.

Duration of atrial fibrillation among patients with history of cerebral thromboembolism

In patients with mitral stenosis, Acarturk et al demonstrated that atrial fibrillation, mitral valve area and left atrial enlargement were independent predictors of spontaneous echo contrast, and left atrial

spontaneous echo contrast was the principal determinant of thromboembolism.[19] Petersen reported that paroxysmal atrial fibrillation was associated with a lower risk of thromboembolic complications than permanent atrial fibrillation.[20] Weigner et al demonstrated that the incidence of cardioversion-related clinical thromboembolism among patients presenting with atrial fibrillation lasting less than 48 hours was low.[21] In our study, we demonstrated that the duration of atrial fibrillation among patients with atrial fibrillation and history of cerebral thromboembolism was significantly longer than that of patients with atrial fibrillation and without history of cerebral thromboembolism. Our results should be viewed as preliminary and await confirmation by larger clinical study.

Study limitations

There were two limitations in this study. First, we did not measure cerebrospinal fluid ET-1 levels in our study; therefore, we do not know whether cerebrospinal fluid ET-1 levels were higher in MS patients with history of cerebral thromboembolism than in patients with atrial fibrillation and without history of cerebral thromboembolism. Second, because the number of patients having history of cerebral thromboembolism was small, we could not completely exclude the possibility of a type 2 error and our results should be viewed as preliminary and await confirmation by larger clinical study.

Conclusions

In patients with MS, the venous plasma ET-1 levels among patients with history of cerebral thromboembolism did not differ from those of patients without history of cerebral thromboembolism. The duration of atrial fibrillation among patients with atrial fibrillation and history of cerebral thromboembolism was significantly longer than that of patients with atrial fibrillation and without history of cerebral thromboembolism.

Acknowledgments

We would like to thank Ms. Hsiu-Chin Tsai for her technical assistance. This study was supported by a grant from Chang Gung Memorial Hospital, Chang Gung University (grant no. CMRP1139).

REFERENCES

1. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411-5.
2. Chen MC, Wu CJ, Yip HK, Chang HW, Chen CJ, Yu TH, Hung WC. Increased circulating endothelin-1 in rheumatic mitral stenosis: irrelevance to left atrial and pulmonary artery pressures. Chest 2004;125:390-6.
3. Yamamoto K, Ikeda U, Mito H, Fujikawa H, Sekiguchi H, Shimada K. Endothelin production in pulmonary circulation of patients with mitral stenosis. Circulation 1994;89: 2093-8.
4. Kinoshita O, Yoshimi H, Nagata S, Ishikura F, Kimura K, Yamabe T, Takagaki K, Miyatake K, Omae T. Rapid increase in plasma endothelin concentrations during percutaneous balloon dilatation of the mitral valve in patients with mitral stenosis. Br Heart J 1993;69:322-6.
5. Yin R, Zhao D, Zhu S, Tao X, Zeng Z, Xia S. Changes of plasma endothelin concentrations before and after percutaneous balloon mitral valvuloplasty in patients with mitral stenosis. Chin Med Sci J 1996;11:78-83.
6. Snopek G, Pogorzelska H, Korewicki J. Influence of valve replacement on plasma endothelin-1 level in mitral stenosis. J Heart Valve Dis 2000;9:82-7.
7. Ziv I, Fleminger G, Djaldetti R, Achiron A, Melamed E, Sokolovsky M. Increased plasma endothelin-1 in acute ischemic stroke. Stroke 1992;23:1014-6.
8. Franceschini R, Gandolfo C, Cataldi A, Del Sette M, Rolandi A, Corsini G, Rolandi E, Barreca T. Twenty-four-hour endothelin secretory pattern in stroke patients. Biomed Pharmacother 2001;55:272-6.
9. Estrada V, Téllez MJ, Moya J, Fernández-Durango R, Egido J, Fernández A. High plasma levels of endothelin-1 and atrial natriuretic peptide in patients with acute ischemic stroke. Am J Hypertens 1994;7:1085-9.
10. Hamann GF, Isenberg E, Strittmatter M, Moili R, Schimrigk K. Big-endothelin in acute ischemic stroke. J Stroke Cerebrovasc Dis 1993;3:256-60.
11. Yair L, Gideon F, Ronit G, Ronit G, Ida SP, Mordechai S. Endothelin in cerebrospinal fluid and plasma of patients in the early stage of ischemic stroke. Stroke 1997;28: 1951-5.
12. Haapaniemi E, Tatlisumak T, Hamel K, Soinne L, Lanni C, Opgenorth TJ, Kaste M. Plasma endothelin-1 levels neither increase nor correlate with neurological scores, stroke risk factors, or outcome in patients with ischemic stroke. Stroke 2000;31:720-5.
13. Anwaar I, Gottsäter A, Lindgärde F, Mattiasson I. Increasing plasma neopterin and persistent plasma endothelin during follow-up after acute cerebral ischemia. Angiology 1999;50:1-8.
14. Lin JJ, Huang CX, Fang CH, Chen F, Jiang H, Tang QZ,

Li GS. Circadian variation in ischemic threshold in patients with stable angina: relation to plasma endothelin-1. Angiology 2002;53:409-13.
15. Eguchi K, Kario K, Shimada K, Mori T, Nii T, Ibaragi K. Circadian variation of blood pressure and neurohumoral factors during the acute phase of stroke. Clin Exp Hypertens 2002;24:109-14.
16. Suzuki N, Matsumoto H, Miyauchi T, Kitada C, Tsuda M, Goto K, Masaki T, Fujino M. Sandwich-enzyme immunoassays for endothelin family peptides. J Cardiovasc Pharmacol 1991;17:S420-S422.
17. Hirata Y, Matsunaga T, Ando K, Furukawa T, Tsukagoshi H, Marumo F. Presence of endothelin-1-like immunoreactivity in human cerebrospinal fluid. Biochem Biophys Res Commun 1990;166:1274-8.
18. Suzuki H, Sato S, Suzuki Y, Oka M, Tsuchiya T, Lino I, Yamanaka T, Ishihara N, Shimoda SI. Endothelin immunoreactivity in cerebrospinal fluid of patients with subarachnoid haemorrhage. Ann Med 1990;22:233-6.
19. Acarturk E, Usal A, Demir M, Akgul F, Ozeren A. Thromboembolism risk in patients with mitral stenosis. Jpn Heart J 1997;38:669-75.
20. Peterson P. Thromboembolic complications in atrial fibrillation. Stroke 1990;21:4-13.
21. Weigner MJ, Caulfield TA, Danias PG, Silverman DI, Manning WJ. Risk for clinical thromboembolism associated with conversion to sinus rhythm in patients with atrial fibrillation lasting less than 48 hours. Ann Intern Med 1997;126:615-20.

風濕性心臟病僧帽瓣狹窄併發腦部血管栓塞之病患其血液中之內皮升壓素之濃度

馬麗娟 陳勉成 張學文[1]

背 景：過去研究顯示，風濕性心臟病僧帽瓣狹窄之病患其血液中之內皮升壓素濃度會增加。然而，風濕性心臟病僧帽瓣狹窄併發腦部血管栓塞者，其血液中之內皮收縮素之濃度則未曾研究過。

方 法：我們研究20位中度或重度風濕性心臟病僧帽瓣狹窄之病患其血液中之內皮升壓素之濃度，其中16位爲永久性心房纖維性振顫，四位爲正常心房節律者。其中6位病患有腦部血管栓塞之病史，其餘14位無任何血管栓塞之病史或證據。我們是以solid phase sandwich enzyme linked-immuno-sorbent assay來測量血液中之內皮升壓素之濃度。

結 果：6位風濕性心臟病僧帽瓣狹窄併發腦部血管栓塞者，其血液中之內皮升壓素之濃度與其他14位無任何腦部血管栓塞病史或證據之病患之血液中內皮升壓素之濃度，無統計學上之差異。

結 論：雖然風濕性心臟病僧帽瓣狹窄之病患其血液中之內皮升壓素之濃度會增加，然而，有腦部血管栓塞病史之患者其血液中之內皮升壓素之濃度未明顯再增加。

(長庚醫誌 2004;27:794-800)

關鍵字：風濕性心臟病僧帽瓣狹窄，內皮升壓素，腦部血管栓塞。

長庚紀念醫院 高雄院區 心臟內科，[1]中山大學生物科學系
受文日期：民國93年2月10日；接受刊載：民國93年9月7日。
索取抽印本處：陳勉成醫師，長庚紀念醫院 心臟內科。高雄縣833鳥松鄉大碑路123號。Tel.: (07)731-7123轉2363; Fax: (07)732-2402; E-mail: chenmien@ms76.hinet.net