Plasma Atrial Natriuretic Peptide Concentration Inversely Correlates With Left Atrial Collagen Volume Fraction in Patients With Atrial Fibrillation

Plasma ANP as a Possible Biochemical Marker to Predict the Outcome of the Maze Procedure

Fumiki Yoshihara, MD,* Toshio Nishikimi, MD,*† Yoshikado Sasako, MD,‡ Jun Hino, Ph.D,* Junjiro Kobayashi, MD,‡ Kenji Minatoya, MD,‡ Ko Bando, MD,‡ Yoshio Kosakai, MD,‡ § Takeshi Horio, MD,‡ Shin-ichi Suga, MD,* Yuhei Kawano, MD,‡ Hiroaki Matsuoka, MD,‡ Chikao Yutani, MD,¶ Hisayuki Matsuo, Ph.D,* Soichiro Kitamura, MD,‡ Tohru Ohe, MD,¶ Kenji Kangawa, Ph.D*

Suita, Mibu, Takarazuka and Okayama, Japan

OBJECTIVES
We hypothesized that the plasma atrial natriuretic peptide (ANP) level reflects atrial degenerative change and may predict the outcome of the maze procedure.

BACKGROUND
Although a larger preoperative left atrial dimension and longer duration of atrial fibrillation (AF) have been reported in patients with persistent AF than in those with sinus rhythm (SR), these individual factors were not enough to predict the outcome of the maze procedure.

METHODS
Preoperative plasma ANP levels were measured in consecutive 62 patients who underwent the Kosakai’s modified maze procedure. Moreover, we performed histological and molecular biological examinations in the resected left atrial tissues.

RESULTS
The preoperative plasma ANP was lower in the AF group (n = 13) than it was in the SR group (n = 49) (p < 0.001). Multiple logistic regression analysis revealed that duration of AF and plasma ANP were independently associated with postoperative cardiac rhythm. Among 41 patients with a higher plasma ANP or shorter duration of AF than the median value, SR was restored in 95% of patients. In contrast, in 21 patients with a lower plasma ANP and a longer duration of AF than the median value, SR was restored only in 48% of patients. Histological examination revealed that the collagen volume in the left atrial tissue was higher in AF than it was in SR and inversely correlated with plasma ANP. In addition, the messenger RNA expressions of ANP, collagen type I and type III were lower in AF than they were in SR.

CONCLUSIONS
These results suggest that a combination of plasma ANP and/or duration of AF may predict the success rate for the maze operation. Advanced atrial degenerative change may result in a decrease of atrial ANP secretion.

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The maze procedure effectively restores atrial fibrillation (AF) to sinus rhythm (SR) and to atrial systole resulting in the disappearance of palpitation, an improvement in hemodynamics and the inhibition of thromboembolism (1–5). However, recent studies revealed that the maze procedure has several problems, which include larger operative invasion due to longer aortic crossclamp time and cardiopulmonary bypass time (6), body fluid retention due to reduced atrial natriuretic peptide (ANP) secretion (7,8) and sick sinus syndrome (9,10). Indeed, there are some patients whose SR is not restored after the maze operation (6). Thus, the time has come to discuss whether there is a desirable indication for the maze procedure before a heart operation.

Previous studies have demonstrated that there might be a low restore rate in patients who have a larger left atrial systolic dimension (LAD) on echocardiography and longer duration of AF (6). However, these individual parameters were not enough to evaluate whether there is a desirable indication for the maze procedure. A noninvasive biochemical marker would be beneficial for this purpose. Atrial natriuretic peptide is mainly synthesized in the atrium, and a previous report suggested that ANP secretion becomes insufficient in remarkable fibrosis in the atrium (11). In

From the *National Cardiovascular Center Research Institute, Suita, Japan; the †Department of Hypertension and Cardiorenal Medicine, Dokkyo University School of Medicine, Mibu, Japan; the ‡Division of Cardiovascular Surgery and Hypertension, National Cardiovascular Center, Suita, Japan; the §Division of Cardiovascular Surgery, Takarazuka Citizen’s Hospital, Takarazuka, Japan; the ¶Division of Pathology, National Cardiovascular Center, Suita, Japan; and the Department of Cardiovascular Medicine, Okayama University Medical School, Okayama, Japan. Supported by the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research (OPSR) of Japan.

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addition, a recent study reported that the patients who had a longer duration of AF were reported to have lower plasma ANP levels (12), suggesting that patients with a longer duration of AF may have reduced ANP secretion presumably due to the advanced atrial degenerative changes. We hypothesized that the plasma ANP levels reflect the atrial qualitative changes and may be a biochemical marker for predicting the outcome of the maze procedure. To test this hypothesis, in the present study we measured the preoperative plasma ANP level in the patients who underwent the maze procedure. Moreover, to explore the underlying mechanism of reduced ANP secretion, we performed histological and molecular biological examinations in the resected left atrial tissues.

**METHODS**

**Patients.** Sixty-two patients who underwent Kosakai’s modified maze procedure were studied. Each subject provided informed consent for participation. All patients with AF, as a result of organic heart disease, underwent the maze procedure as previously reported (6). On the basis of the postoperative rhythm (duration after surgery: 31.2 ± 20.7 months), the subjects were divided into the SR group (n = 49) or the AF group (n = 13).

**Measurements of plasma ANP levels.** Venous blood was withdrawn at one day before operation. Immunoradiometric assay was performed to measure plasma ANP levels (Shiono RIA ANP kit; Shionogi Co., Ltd., Osaka, Japan) as previously reported (13).

**Histological examination.** Sections 5 μm thick of paraffinized left atrial appendage tissues were stained with Masson’s trichrome to highlight collagen fibers. The collagen volume fraction was determined by measuring the area of stained tissue within a given field (14). In the resected left atrial appendage, 10 fields were analyzed. We evaluated 30 randomly selected patients (SR group: n = 24, AF group: n = 6).

**Messenger RNA (mRNA) expressions of ANP, collagen type I and type III in left atrial appendage tissue.** To obtain molecular biological evidence regarding the atrial qualitative differences between the AF and SR groups, resected left atrial tissues were frozen in liquid nitrogen and stored at −80°C for Northern blot analysis in 31 patients (SR group: n = 26, AF group: n = 5). A Pst I restriction fragment of human ANP complementary DNA (cDNA) corresponding to nucleotides −100 to 589 (15) was used as the human ANP cDNA probe. The human collagen type I and type III cDNA probes were synthesized by polymerase chain reaction using the primers: collagen type I sense, 5′-CAAGGTTGGTTGCGATGACG-3′; collagen type I antisense, 5′-ATTCCCTCGGTGATTCTC-3′; collagen type III sense, 5′-ATCCGTTCTGCG-GATGACATAAAT-3′; collagen type III antisense, 5′-GCCTGGAGTCCTCCTACTGCTACT-3′. RNA extraction and Northern blot analyses were done using a procedure previously described (15–17).

**Statistics.** Values are the mean ± SD. Differences in proportions were evaluated by chi-square analysis, unpaired t test for continuous normally distributed variables and Mann-Whitney U test for nonnormally distributed variables. The factors that related at the p < 0.1 level were selected as independent variable candidates for multiple logistic regression analyses, which were used to evaluate the independent contribution of clinical parameters to cardiac rhythm after the operation. Differences were considered to be statistically significant when the p value was < 0.05. Correlation coefficients were calculated using linear regression analysis.

**RESULTS**

**Plasma ANP levels in the AF and SR groups.** There were no significant differences in age, gender, body surface area, underlying heart diseases, operations or cardiothoracic ratio (CTR) between the two groups. The LAD, the duration of AF and preoperative plasma ANP level were significantly larger, longer and lower in the AF group than they were in the SR group (Table 1).

**Histological examination data.** The collagen volume fraction in the left atrial appendage tissue was higher in the AF group than it was in the SR group (Fig. 1). The collagen volume fraction correlated negatively with the plasma ANP levels (R = −0.39, p < 0.05) (Fig. 2A) and positively with the duration of AF (R = 0.62, p < 0.001) (Fig. 2B).

**The effects of plasma ANP and duration of AF on the postoperative cardiac rhythm.** Table 2 shows the results of the multiple logistic regression analyses to assess the factors independently contributing to the cardiac rhythm after the operation (mean duration after surgery: 31.2 ± 20.7 months). The LAD, duration of AF, CTR and plasma ANP levels were added to this model. According to the multiple logistic regression analyses, only the duration of AF and plasma ANP levels were independent predictors of cardiac rhythm after the maze operation. In 31 patients with a higher plasma ANP than the median value (>40 pg/ml), SR was restored in 30 (97%) (Fig. 3). In 28 patients with a shorter duration of AF than the median value (<4.0 years), SR was restored in 27 (96%) (Fig. 3). In contrast, in 21 patients with a lower or equal plasma ANP (≤40 pg/ml) and with a longer or equal duration of AF (≥4.0 years), SR was restored only in 10 (48%) (Fig. 3).
Combined assessment of plasma ANP level and duration of AF versus duration of AF alone: prediction of persistent AF after the maze operation. Table 3 shows the sensitivity, specificity, accuracy and positive and negative predictive value of the combined assessment of plasma ANP level and duration of AF and the duration of AF alone for predicting persistent AF after the maze operation. The combined assessment had a significantly greater specificity and accuracy than the duration of AF alone (80% vs. 55%, 81% vs. 63%, p < 0.05, respectively), and there was a trend toward an increase in positive predictive value for predicting persistent AF after the operation.

The mRNA expression of ANP and collagen. As the result of plasma ANP levels, the mRNA expression of ANP in the left atrial appendage tissues was also significantly lower (p < 0.05) in the AF than it was in the SR (Fig. 4). In contrast with the higher collagen volume fraction in AF than in SR, the mRNA expressions of type I and III collagen in the left atrial appendages were lower (both: p < 0.01) in the AF group than they were in the SR group (Fig. 4).

DISCUSSION

Comparison with other studies. An earlier study reported that there were significant differences in LAD as determined by echocardiography and duration of AF between the SR and AF groups after the maze procedure (6). In addition, recent studies also demonstrated that duration of AF, LAD and CTR were preoperative risk factors for persistent AF after the maze operation (18–20). In the present study, the patients with persistent AF after the maze procedure had a longer history of AF and larger LAD compared with the SR group. These results were consistent with the previous studies and suggest that these parameters might be useful for predicting the outcome of the maze procedure. However, since these parameters had considerable overlap between the AF and SR groups and none had

![Figure 1. Masson’s trichrome staining of the excised left atrial appendages under light microscopy and quantification of the collagen volume fraction in the atrial fibrillation (AF) and sinus rhythm (SR) groups.](image-url)
cut-off points, it seems to be difficult to determine a preoperative indication for the maze procedure using these individual parameters.

**Plasma ANP as information for predicting the outcome of the maze procedure.** In the present study, we measured preoperative plasma ANP levels in patients who underwent the maze procedure. Atrial natriuretic peptide is mainly synthesized in the atrium, and its secretion is reduced in remarkable fibrosis in the atrium (12). Therefore, we hypothesized that plasma ANP reflects the atrial myocardial qualitative change and might be beneficial for predicting the outcome of the maze procedure. In the present study, patients with a higher preoperative plasma ANP level (>40 pg/ml) than the median value showed a high SR restoration rate (97%), suggesting that high plasma ANP levels may be a noninvasive biochemical marker for success in the maze operation. In addition, to assess the factors independently contributing to the cardiac rhythm after the operation, multiple logistic regression analyses were performed. According to these analyses, only duration of AF and plasma ANP level were independent predictors of cardiac rhythm after the maze operation. The significant relationship between the outcome of the maze procedure and the duration of AF was consistent with previous reports (6,20). In fact, patients with a shorter duration of AF (<4.0 years) than the median value, irrespective of their ANP levels, showed a high SR restoration rate, suggesting that a short duration of AF is also a marker for success in the maze operation. Furthermore, the combined assessment of plasma ANP level and duration of AF had a significantly greater specificity and accuracy and a trend toward an increase in positive predictive value compared with the duration of AF alone for predicting the outcome of the maze operation. In fact, patients with lower plasma ANP levels (<40 pg/ml) and a longer duration of AF (≥4.0 years) showed a low restoration rate (48%), suggesting that the combination of a low plasma ANP level and long duration of AF is predictive of a low success rate for the maze procedure. Specifically, in the patients with high ANP levels (>50 pg/ml), SR was completely restored in 29/29 (100%). Furthermore, among the patients with a lower ANP (<40 pg/ml) and with a longer duration of AF history (≥8.0 years), SR was restored only in 3/12 (25%). Thus, a high ANP level or short duration of AF may predict a good outcome for the maze procedure, whereas a low ANP with long duration of AF may predict a lower rate of recovery to SR after the maze procedure. Since recent studies revealed that the maze procedure has several problems, which include greater operative invasion (6), body fluid retention and sick sinus syndrome (9,10), these parameters may help to determine an indication for the maze procedure.

**Table 2.** Associations Between Cardiac Rhythm After the Operation and Clinical Parameters by Multiple Logistic Regression Analyses

<table>
<thead>
<tr>
<th>Cardiac Rhythm After the Operation</th>
<th>Chi-Square</th>
<th>df</th>
<th>p Value</th>
</tr>
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<tbody>
<tr>
<td>LAD</td>
<td>1.190</td>
<td>1</td>
<td>0.275</td>
</tr>
<tr>
<td>Duration of AF</td>
<td>4.717</td>
<td>1</td>
<td>0.030</td>
</tr>
<tr>
<td>CTR</td>
<td>0.0002</td>
<td>1</td>
<td>0.988</td>
</tr>
<tr>
<td>Plasma ANP</td>
<td>8.300</td>
<td>1</td>
<td>0.004</td>
</tr>
</tbody>
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AF = atrial fibrillation; ANP = atrial natriuretic peptide; CTR = cardiothoracic ratio; df = degrees of freedom; LAD = left atrial dimension.
Underlying mechanism for reduced ANP in patients with AF. A previous study reported that plasma ANP levels were increased due to an atrial pressure elevation in patients with supraventricular tachycardia and AF (21) and promptly decreased after electrical cardioversion (22). Furthermore, patients who had an increase in the plasma ANP levels from rest to peak exercise before defibrillation had a better outcome of electrical cardioversion (23). In addition, atrial mechanical overload leads to an atrial qualitative change resulting in the initiation and perpetuation of AF (24–26). These findings suggest that patients with maintaining SR after electrical cardioversion may have less atrial qualitative change and that the ability of ANP secretion was reserved in these patients. In addition, previous study demonstrated that the plasma ANP levels were lower among patients with AF of longer duration (11). This finding suggests that the plasma ANP level also reflects the qualitative change of atrial myocardium. However, there was no direct evidence, which clarified the relationship between plasma ANP levels and histological findings. In the present study, we performed a histological examination in the excised left atrial appendage tissues and demonstrated for the first time that the collagen volume fraction was significantly increased in the AF group compared with the SR group and that the

**Table 3. Predictive Values Associating the Combination of ANP and Duration of AF and the Duration of AF Alone With the Outcome of the Maze Operation**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of AF</td>
<td>12/13 (92%)</td>
<td>27/49 (55%)</td>
<td>39/62 (63%)</td>
<td>12/34 (35%)</td>
<td>27/28 (96%)</td>
</tr>
<tr>
<td>Plasma ANP and</td>
<td>11/13 (84%)</td>
<td>39/49 (80%)</td>
<td>50/62 (81%)</td>
<td>11/21 (52%)</td>
<td>39/41 (95%)</td>
</tr>
<tr>
<td>duration of AF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fisher’s p value</td>
<td>&gt;0.9999</td>
<td>0.0171</td>
<td>0.0452</td>
<td>0.2658</td>
<td>&gt;0.9999</td>
</tr>
</tbody>
</table>

Cut-off values were 4.0 years and 40 pg/ml for AF duration and plasma ANP, respectively.

AF = atrial fibrillation; ANP = atrial natriuretic peptide.

**Figure 4.** Representative Northern blots showing collagen type I, type III and atrial natriuretic peptide (ANP) in the excised left atrial appendages in patients with persistent atrial fibrillation after the maze procedure (AF group) and patients with sinus rhythm (SR group). Bar graph shows the summary of findings of Northern blot analysis of atrial collagen type I, type III and ANP messenger RNA in the AF and SR groups.
collagen volume fraction negatively correlated with the plasma ANP level. Furthermore, we showed that the ANP mRNA expression was significantly decreased in the AF group compared with the SR group. Thus, the ability to secrete ANP was reduced in the AF group presumably due to the advanced histological and molecular biological changes in the left atrial myocardium. Plasma ANP levels, therefore, may reflect atrial qualitative change and provide information for predicting the outcome of the maze procedure. We also detected the reduced mRNA expressions of collagen type I and III, although the collagen volume fraction was significantly higher in left atrial appendage in the AF group compared with the SR group. Thus, the ability to resolve these questions.

Study limitations. There were relatively small numbers of patients to prove the relationship between atrial degenerative changes and plasma ANP levels in the present study. Furthermore, we could not completely deny the possibility that the reason for remaining AF may not only be due to the advanced atrial degenerative change but also to the technical difficulties of maze procedure in large atria. Further study is required in a large number of patients in the future to resolve these questions.

Conclusions. A high preoperative plasma ANP level or short duration of AF may predict a high success rate for the maze procedure, whereas a low plasma ANP level with longer duration of AF may predict a low success rate.

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Reprint requests and correspondence: Dr. Toshio Nishikimi, Department of Hypertension and Cardiorenal Medicine, Dokkyo University School of Medicine, Mibu, Tochigi 321-0293, Japan. E-mail: nishikim@dokkyomed.ac.jp.

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