Intracoronary Acetylcholine Provocation Testing
– Omission of the 20-μg Dose Is Feasible in Patients Without Coronary Artery Spasm in the Other Coronary Artery –
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Background: Based on the Japanese Circulation Society guideline of vasospastic angina, incremental doses of acetylcholine (ACh) are prescribed for coronary spasm provocation: 20 and 50 μg for the right coronary artery (RCA), and 20, 50, and 100 μg for the left coronary artery (LCA). However, the requirement for each dose of ACh has not been fully evaluated.

Methods and Results: A total of 249 patients who underwent ACh provocation test for both the RCA and LCA were included. The positive diagnosis of intracoronary ACh provocation test was defined as total or subtotal coronary artery narrowing accompanied by chest pain and/or ischemic ECG changes. Positive ACh provocation test was observed in 116 patients (47%). Patients without vasospasm in the LCA had a lower incidence of vasospasm in the RCA induced by 20 μg of ACh compared with those with vasospasm in LCA (0.8% vs. 27.5%, P<0.001). Similarly, vasospasm in the LCA induced by 20 μg of ACh was observed less frequently in patients without than with vasospasm in the RCA (6.1% vs. 26.7%, P<0.001). In all patients without vasospasm in the other coronary artery, intracoronary administration of 50 μg of ACh was performed without any complications.

Conclusions: Skipping the provocation test with 20 μg of ACh in patients without coronary artery spasm in the other coronary artery may be possible. (Circ J 2016; 80: 1820–1823)

Key Words: Acetylcholine provocation test; Diagnostic tests; Vasospastic angina

Vasospastic angina (VA) is an important cardiac disorder that causes myocardial infarction, ventricular arrhythmia, or sudden cardiac arrest.1–3 Although the intracoronary acetylcholine (ACh) provocation test is useful for diagnosing VA,4,5 there are various protocols in terms of the doses of ACh.4,6 Based on the guidelines of the Japanese Circulation Society for the diagnosis and treatment of patients with VA, incremental doses of ACh are prescribed for the coronary artery vasospasm provocation test: 20 and 50 μg for the right coronary artery (RCA), and 20, 50, and 100 μg for the left coronary artery (LCA).8 However, the requirement for each dose of ACh to provoke coronary artery vasospasm has not been fully evaluated.

Methods
Study Population
A total of 269 consecutive patients underwent the intracoronary ACh provocation test from April 2012 to December 2014 at Chiba University Hospital. Patients who underwent the ACh provocation test only for the RCA (n=2) or LCA (n=15) were excluded. Patients who received a single dose of 10 μg of ACh for the RCA (n=1) and 100 μg for the LCA (n=2) were also excluded. Thus, 249 patients who underwent the ACh provocation test for both the RCA and LCA were included in this study. Written informed consent for examination was given by all patients, and the Ethics Committee of Chiba University approved this study.

Intracoronary ACh Provocation Test
Intracoronary ACh provocation tests were performed according to the guidelines of the Japanese Circulation Society Joint Working Group for the diagnosis and treatment in the clinical setting of patients with VA.8 In elective cases, all vasodilators, such as calcium-channel blockers and long-acting nitrates, were discontinued at least 48 h before the examination, except for sublingual nitroglycerin as needed. After insertion of a temporary pacing electrode in the right ventricle, control angiography
Skipping Provocation With 20\( \mu \)g ACh

Table 1. Characteristics of the Study Patients Undergoing the ACh Provocation Test (n=249)

| Characteristic                        | Value                |
|---------------------------------------|----------------------|
| Age (years)                           | 63.6±11.7            |
| Men                                   | 138 (55%)            |
| Body mass index (kg/m\(^2\))          | 24.0±4.5             |
| Hypertension                          | 164 (66%)            |
| Diabetes mellitus                     | 52 (21%)             |
| Dyslipidemia                          | 180 (72%)            |
| Current smoker                        | 52 (21%)             |
| Prior myocardial infarction           | 32 (14%)             |
| Rest angina                           | 173 (69%)            |
| Effort angina                         | 8 (3%)               |
| Rest and effort angina                | 52 (21%)             |
| No angina                             | 16 (6%)              |
| Calcium-channel blocker               | 121 (49%)            |
| Long-acting nitrate                   | 46 (19%)             |
| Antiplatelet drug                     | 84 (34%)             |
| Statin                                | 94 (38%)             |
| ACEI or ARB                           | 91 (37%)             |
| \( \beta \)-blocker                   | 37 (15%)             |

ACEI, angiotensin-converting enzyme inhibitor; ACh, acetylcholine; ARB, angiotensin-receptor blocker.

Table 2. ACh Provocation Test (n=249)

| Initial artery of ACh provocation       | Value |
|-----------------------------------------|-------|
| Right coronary artery                   | 16 (6%) |
| Left coronary artery                    | 233 (94%) |
| No. of vessels with vasospasm           |       |
| 0                                       | 108 (43%) |
| 1                                       | 68 (27%) |
| 2                                       | 53 (21%) |
| 3                                       | 20 (8%)  |
| Multivessel spasm                       | 73 (29%) |
| Provoked coronary artery                 |       |
| Right                                   | 86 (36%) |
| Left anterior descending                | 114 (46%) |
| Left circumflex                         | 34 (14%) |
| Evidence of ischemia                    |       |
| ECG change                              | 83 (33%) |
| Chest pain                              | 108 (43%) |
| Positive ACh provocation test           | 116 (47%) |

ACh, acetylcholine.

Table 3. Cardiac Complications of Study Patients Undergoing the ACh Provocation Test

| Complication                      | Left coronary artery | Right coronary artery |
|-----------------------------------|----------------------|-----------------------|
|                                   | 20 \( \mu \)g | 50 \( \mu \)g | 100 \( \mu \)g | 20 \( \mu \)g | 50 \( \mu \)g |
| Death                             | 0           | 0           | 0           | 0           | 0           |
| Cardiogenic shock                 | 0           | 0           | 1 (0.5%)   | 0           | 0           |
| Ventricular tachycardia           | 0           | 0           | 1 (0.5%)   | 0           | 1 (0.6%)   |

ACh, acetylcholine.

of the LCA and RCA was performed. ACh was injected in incremental doses of 20 and 50 \( \mu \)g into the RCA, and 20, 50 and 100 \( \mu \)g into the LCA over a period of 20 s. Coronary angiography (CAG) was performed 1 min after the start of each injection. In the event of an ischemic change on ECG or chest pain, CAG was performed at that time. Doses of ACh were given at 5-min intervals. After the ACh provocation test, 1 mg of isosorbide dinitrate was administered into the RCA and LCA, and CAG was then performed.

Definitions

Angiographic coronary artery vasospasm was defined as total or subtotal occlusion induced by the ACh provocation test. It was evaluated by 2 experienced cardiologists, who were unaware of the patients’ clinical characteristics. A positive diagnosis was defined as angiographic coronary artery vasospasm accompanied by chest pain and/or ischemic ECG changes, such as transient ST elevation \( \geq 0.1 \) mV, ST depression \( \geq 0.1 \) mV, or new appearance of negative U waves, recorded in at least 2 contiguous leads on the 12-lead ECG. Multivessel spasm was defined as ACh-induced coronary artery vasospasm in \( \geq 2 \) major epicardial arteries. Cardiac complications were defined as death, ventricular fibrillation or tachycardia, and cardiogenic shock by ACh provocation test.

Statistical Analysis

Statistical analysis was performed with SAS statistical software package version 9.4 (SAS Institute, Cary, NC, USA). Data are expressed as mean±SD or frequency (%). Categorical variables were compared with Fisher’s exact test. A value of P<0.05 was considered significant.

Results

Table 1 presents the patients’ baseline characteristics. There were 91 patients with ECG changes and 126 patients with chest symptoms during the ACh provocation test. In these patients, 83 (91%) and 108 patients (86%) were accompanied by angiographic vasospasm. A positive ACh provocation test was observed in 116 patients (47%) (Table 2). Table 3 shows cardiac complications associated with ACh provocation tests. Although 1 case of ventricular tachycardia was documented in each of the LCA and RCA, both spontaneously terminated. Cardiogenic shock occurred in 1 patient by ACh provocation of the LCA, caused by diffuse severe vasospasm at both the left descending and circumflex arteries with rapid lowering of systolic blood pressure to 50 mmHg, followed by inevitable intravenous administration of noradrenaline.

Patients without vasospasm in the LCA had a lower incidence of vasospasm in the RCA induced by 20 \( \mu \)g of ACh compared with those with vasospasm in the LCA (0.8% vs. 27.5%, P<0.001) (Figure 1). In all patients without vasospasm in the LCA, intracoronary administration of 50 \( \mu \)g of ACh was performed, regardless of vasospasm induced by 20 \( \mu \)g of ACh.
in the RCA, without any complications associated with administration of ACh. Similarly, vasospasm in the LCA induced by 20μg of ACh was observed less frequently in patients without than with vasospasm in the RCA (6.1% vs. 26.7%, P<0.001) (Figure 2). In addition, the incidence of vasospasm in the RCA by 20μg of ACh in patients without vasospasm in the LCA was significantly lower than that of vasospasm in the LCA by 20μg of ACh in patients without vasospasm in the RCA (0.8% vs. 6.1%, P=0.03).

In all patients without vasospasm in the RCA, the intracoronary administration of 50μg of ACh was performed, regardless of vasospasm induced by 20μg of ACh in the LCA, without any complications associated with administration of ACh.

**Discussion**

The main finding of the present study was that vasospasm was induced infrequently by administration of 20μg of ACh in patients without coronary artery spasm in the other coronary artery, especially in patients without vasospasm in the LCA. In patients with a positive provocation test with 20μg of ACh
and no vasospasm in the other coronary artery, provocation test with 50μg of ACh was performed without any complications. Thus, ACh provocation probably should be performed initially for the LCA according to the guidelines by Japanese Circulation Society, leading to skipping of the provocation test with 20μg of ACh for the RCA to reduce contrast volume and procedure time in cases of no vasospasm in the LCA.

**ACh Provocation Doses**

The intracoronary ACh provocation test is a validated method of diagnosing VA, with few serious complications. A variety of protocols, in terms of ACh doses, have been used, but there are few reports evaluating these doses of ACh. Although the guidelines of the Japanese Circulation Society for the diagnosis and treatment of patients with VA recommend administering incremental doses of ACh, sometimes that is not done in Japanese clinical settings. Sueda et al. showed that 16% of the hospitals used a single dose of ACh for each coronary artery (ie, 50μg for the RCA and 100μg for the LCA). Furthermore, the usefulness of higher doses of ACh such as 80μg for the RCA and 200μg for the LCA has recently been demonstrated. In the present study, 2 cases of ventricular tachycardia and one of cardiogenic shock were documented, and all of them were induced by the maximum dose of ACh for each of the coronary arteries (LCA and RCA). Therefore, it is suggested that the first target artery should always be examined with incremental doses of ACh to avoid serious complications, because the provocation test by the incremental doses may be able to detect the threshold of vasospastic activity in individual patients.

Multivessel coronary spasm is a prognostic factor of patients with vasospasm. On the other hand, previous studies have shown relatively low disease activity in patients without vasospasm by provocation test in either coronary artery. Thus, a provocation test with 50μg of ACh as the first dose may be performed safely in patients without vasospasm in the other coronary artery.

In patients with vasospasm in the other coronary artery, it might also be possible that a provocation test with 50μg of ACh as the first dose could be safely performed. In the present study population, however, 1 patient with vasospasm in the LCA developed ventricular tachycardia after provocation with 50μg of ACh in the RCA. In addition, 2 of the 86 patients with vasospasm in the LCA and 6 of the 120 patients with vasospasm in the RCA did not receive intracoronary administration of 50μg of ACh in the other coronary artery, because sufficient provocation was achieved by 20μg of ACh. Therefore, it is still unclear whether the provocation test with 50μg of ACh as the first dose can be safely conducted in patients with vasospasm in the other coronary artery.

**Study Limitations**

First, the number of patients was relatively small. Second, patients with a provocation test for only one coronary artery were excluded. Third, intracoronary ACh provocation tests were not performed in the morning in any of the patients, especially those in an unstable condition. There is a circadian variation in the occurrence of coronary vasospasm. Further investigation is required for this issue.

**Conclusions**

During VA evaluation, patients without coronary artery spasm in the other coronary artery may be able to skip the provocation test of 20μg of ACh, thereby using less contrast and shortening the overall procedure time.

**Disclosure**

None.

**References**

1. Takagi Y, Yasuda S, Takahashi J, Takeda M, Nakayama M, Ito K, et al. Importance of dual induction tests for coronary vasospasm and ventricular fibrillation in patients surviving out-of-hospital cardiac arrest. *Circ J* 2009; 73: 767–769.
2. Ong P, Athanasiadis A, Borgulya G, Voehringer M, Sechtem U. 3-year follow-up of patients with coronary artery spasm as cause of acute coronary syndrome: The CASPAr (coronary artery spasm in patients with acute coronary syndrome) study follow-up. *J Am Coll Cardiol* 2011; 57: 147–152.
3. Ong P, Aziz A, Hansen HS, Prescott E, Athanasiadis A, Sechtem U. Structural and functional coronary artery abnormalities in patients with vasospastic angina pectoris. *Circ J* 2015; 79: 1431–1438.
4. Yusae H, Horio Y, Nakamura N, Fujii H, Imoto N, Sonoda R, et al. Induction of coronary artery spasm by acetylcholine in patients with variant angina: Possible role of the parasympathetic nervous system in the pathogenesis of coronary artery spasm. *Circulation* 1986; 74: 955–963.
5. Sueda S, Ochi N, Kawada H, Matsuem S, Hayashi Y, Tsuruoka T, et al. Frequency of provoked coronary vasospasm in patients undergoing coronary arteriography with spasm provocation test of acetylcholine. *Am J Cardiol* 1999; 83: 1186–1190.
6. Sueda S, Itoz Y, Kohn H, Fukuda H, Uraoka T. Need for documentation of guidelines for coronary artery spasm: An investigation by questionnaire in Japan. *Circ J* 2005; 69: 1333–1337.
7. Ong P, Athanasiadis A, Borgulya G, Mahholdt H, Kaski JC, Sechtem U. High prevalence of a pathological response to acetylcholine testing in patients with stable angina pectoris and unobstructed coronary arteries: The ACOVA Study (Abnormal Coronary Vasomotion in patients with stable angina and unobstructed coronary arteries). *J Am Coll Cardiol* 2012; 59: 655–662.
8. JCS Joint Working Group. Guidelines for diagnosis and treatment of patients with vasospastic angina (coronary spastic angina) (JCS 2013): Digest version. *Circ J* 2014; 78: 2779–2801.
9. Okumura K, Yasue H, Matsuyama K, Goto K, Miyagi H, Ogawa H, et al. Sensitivity and specificity of intracoronary injection of acetylcholine for the induction of coronary artery spasm. *J Am Coll Cardiol* 1988; 12: 883–888.
10. Ong P, Athanasiadis A, Borgulya G, Voksli I, Bastaiaen R, Kubik S, et al. Clinical usefulness, angiographic characteristics, and safety evaluation of intracoronary acetylcholine provocation testing among 921 consecutive white patients with unobstructed coronary arteries. *Circulation* 2014; 130: 1723–1730.
11. Sueda S, Kohn H, Ochi T, Uraoka T. Overview of the acetylcholine spasm provocation test. *Clin Cardiol* 2015; 38: 430–438.
12. Sueda S, Kohn H, Miyoshi T, Sakaue T, Sasaki Y, Habara H. Maximal acetylcholine dose of 200μg into the left coronary artery as a spasm provocation test: Comparison with 100μg of acetylcholine. *Heart Vessels* 2015; 30: 771–778.
13. Takagi Y, Yasuda S, Takahashi J, Tsunoda R, Ogata Y, Seki A, et al. Clinical implications of provocation tests for coronary artery spasm: Safety, arrhythmic complications, and prognostic impact. Multicentre registry study of the Japanese Coronary Spasm Association. *Eur Heart J* 2013; 34: 258–267.
14. Takagi Y, Takahashi J, Yasuda S, Miyata S, Tsunoda R, Ogata Y, et al. Prognostic stratification of patients with vasospastic angina: A comprehensive clinical risk score developed by the Japanese Coronary Spasm Association. *J Am Coll Cardiol* 2013; 62: 1144–1153.
15. Seo SM, Kim PJ, Shin DI, Kim TH, Kim CJ, Min JS, et al. Persistent coronary artery spasm documented by follow-up coronary angiography in patients with symptomatic remission of variant angina. *Heart Vessels* 2013; 28: 301–306.
16. el-Tamimi H, Mansour M, Pepine CJ, Wargovich TJ, Chen H. Circadian variation in coronary tone in patients with stable angina: Protective role of the endothelium. *Circulation* 1995; 92: 3201–3205.
17. Nihel T, Takahashi J, Tsurubaya R, Ito Y, Shiroto T, Hao K, et al. Circadian variation of Rho-kinase activity in circulating leukocytes of patients with vasospastic angina. *Circ J* 2014; 78: 1183–1190.