Endogenous Adenosine May Be Related to Left Ventricular Dysfunction, Dilation, and Wall Thinning in Patients With Heart Disease

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Background: The role of endogenous adenosine in cardiac patients is still unclear, so we investigated the relationship between the plasma adenosine concentration and left ventricular (LV) function, LV dilation and LV wall thinning in cardiac patients.

Methods and Results: In 97 cardiac patients, with angina pectoris, old myocardial infarction, dilated or hypertrophic cardiomyopathy, and valvular heart disease, plasma adenosine concentrations were measured using the LC-MS/MS system, and the LV function, LV end-diastolic dimension (LVDd), LV posterior wall thickness (LVPWth), and interventricular septum thickness (IVSth) were assessed by echocardiography. The plasma adenosine concentration was significantly higher in patients with a LV ejection fraction (EF), an indicator of the LV systolic function, <47% compared with those with LVEF ≥47% (P=0.027). There was no difference between the plasma adenosine concentration and E/e’, an indicator of LV diastolic function. The plasma adenosine concentration was significantly higher in patients with LVDd ≥50 mm than in those with LVDd <50 mm (P=0.030). The plasma adenosine concentration was inversely correlated with IVSth (P=0.003) and LVPWth (P=0.0007). The plasma adenosine concentration was significantly higher in patients with IVSth <8 mm than in those with IVSth ≥8mm (P=0.015), and was significantly higher in patients with LVPWth <8 mm than in those with LVPWth ≥8 mm (P=0.020).

Conclusions: Endogenous adenosine may be related to LV dysfunction, dilation, and wall thinning in cardiac patients.

Key Words: Interventricular septum thickness; LV dysfunction; LV end-diastolic dimension; LV wall thickness; Plasma adenosine concentration

Adenosine has been reported as an agent that triggers an ischemic preconditioning effect on the heart, and the administration of adenosine or an adenosine agonist before prolonged ischemia protects the heart and reduces the size of the myocardial infarct. We previously reported that cilostazole, an antiplatelet agent, reduces the myocardial infarct size, an effect that was blocked by pretreatment with 8-SPT, an adenosine receptor blocker, suggesting that adenosine is involved in the protective effect of cilostazole. An adenosine A1 receptor agonist has been reported to improve left ventricular (LV) function and remodeling in dogs. Furthermore, an adenosine A1 receptor agonist has been reported to attenuate cardiac hypertrophy and prevent heart failure (HF) in a murine model of LV pressure-overload. These reports suggest that adenosine is a cardioprotective agent. The plasma adenosine concentration has been reported as elevated in patients with chronic HF. On the other hand, it was reported that there was no significant difference in plasma adenosine levels between normal subjects and patients with chronic HF of NYHA class III, and that the plasma adenosine level after dynamic exercise was significantly lower in patients with chronic HF than in normal subjects, suggesting that the production of adenosine in response to dynamic exercise was decreased in patients with chronic HF. Therefore, controversy remains on whether the plasma adenosine concentration is elevated in patients with chronic HF. Furthermore, the...
possible contribution of plasma adenosine to LV function, LV dilation and LV wall thinning has not yet been clarified in patients with chronic HF. Therefore, in the present study, we aimed to investigate the relationship between the plasma adenosine concentration and LV function or LV remodeling (LV chamber dilatation and LV posterior wall thickness [LVPWth]) and interventricular septum thickness (IVSth)) in patients with ischemic and non-ischemic heart diseases.

Methods

The protocol of the present study was approved by the ethics committee of Gifu University Graduate School of Medicine (Approval no. 24-368). All patients gave written informed consent before the study commenced. The investigation conformed to the principles outlined in the Declaration of Helsinki (BMJ 1964; ii: 177). The public and trial registry number is R000026665.

Study Patients

We included 97 patients with cardiac diseases, such as angina pectoris (AP), old myocardial infarction (MI), dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), and valvular heart disease (VHD), who were being treated at the outpatient clinic or admitted to Gifu University Hospital. The study period was from July 2016 to June 2017. The enrollment of patients was performed consecutively only for patients who provided written informed consent. The control group (n=11) consisted of normal healthy subjects who visited the outpatient clinic of Gifu University Hospital complaining of general malaise, but after careful examination, were diagnosed as being free from disease with normal echocardiographic findings (mean age: 73.8±5.2, male: 7, female: 4).

LV Function, LV Dilation and LV Wall Thinning

The LV ejection fraction (LVEF: LV systolic function), E/e'(LV diastolic function), LV end-diastolic dimension (LVDd), IVSth, and LVPWth were obtained by echocardiography (iE33, PHILIPS, Tokyo, Japan). The LV ejection fraction (LVEF: LV systolic function), E/e'(LV diastolic function), LV end-diastolic dimension (LVDd), IVSth, and LVPWth were obtained by echocardiography (iE33, PHILIPS, Tokyo, Japan).

Blood Collection and Measurement of Plasma Adenosine Concentration

Blood samples were taken from the antecubital vein and collected into sterile tubes containing EDTA, immediately placed on ice, and then centrifuged at 10,000 g for 15 min. Plasma was then collected and frozen at −83°C until further analysis of plasma adenosine concentrations. Blood samples are conventionally collected in tubes containing dipyridamole 0.02% solution (500 mmol/L), 2'-deoxycoformycin 0.1 ng/mL (20 µL), and EDTA 500 mmol/L for 1 mL of the same blood from the same subjects as in the A method.

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that the blood collection method without dipyridamole and 2'-deoxycoformycin gave relatively accurate levels of adenosine in plasma. Therefore, in the present study, we used tubes containing only EDTA to collect blood samples and measured the plasma adenosine concentration using the method described next.10

The plasma adenosine concentration was measured using a Prominence HPLC system (Shimadzu, Japan) equipped with a 3200 QTRAP MS/MS system with a Turbo V source and an ESI probe (AB SCIEX, Canada). The hydrophilic interaction chromatographic (HILIC) separation was performed on a Tosoh TSKgel Amide-80 column (3μm, 150×2.0 mm i.d.) with a mobile phase consisting of water and acetonitrile (linear gradient: 100–90% acetonitrile over 12 min followed by 90–30% over 8 min) at a flow rate of 0.2 mL/min at 40°C. We recently developed the ESI-MS/MS method for the determination of adenosine in human plasma combined with HILIC separation after simple pretreatment consisting of deproteinization and ultrafiltration.10 This method provides highly sensitive and reproducible multiple-reaction monitoring signals of adenosine (m/z: 268.1/136.1) and 15N5-adenosine (m/z: 273.1/141.1) as an internal standard under positive ESI conditions without ionic suppression caused by matrix compounds in human plasma. The calibration curve was linear across the examined dynamic range from 10 to 500 nmol/L (r=1.000). The relative standard deviations of the MS/MS responses in 6 trials were 2.17%, 2.71%, 2.24%, and 1.82% for plasma samples containing 10, 50, 100, and 500 nmol/L adenosine, respectively. The detailed analytical procedures and validation for the ESI-MS/MS method have been previously published.10

**Blood Biochemical Analysis**
Using the blood samples taken from the antecubital vein, peripheral blood cell counts, a hemogram, and blood biochemical analysis for factors such as creatine kinase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatinine, blood urea nitrogen, C-reactive protein, hemoglobin A1c, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and B-type natriuretic peptide (BNP; Shionoria BNP RIA kit; Shionogi, Osaka, Japan) were performed.

**Drugs Used and Complications**
Drugs used and complications in patients were examined.

**Statistical Analysis**
The data are shown as the mean±1 standard deviation. Categorical data were converted to percentages and compared with a chi-square test or Fisher’s exact test. The normality of data distributions was tested using the Kolmogorov-Smirnov test. The significance of the differences between groups for variables that were normally distributed was determined by an unpaired Student’s t-test.
Relationship Between Plasma Adenosine Concentration and Age, Sex, Hypertension, Diabetes Mellitus, and Hyperlipidemia

There was no relationship between the plasma adenosine concentration and age (Figure 3A). There was no difference in plasma adenosine concentrations between males and females (Figure 3B), between patients with and without hypertension (Figure 3C), between patients with and without diabetes mellitus (Figure 3D), or between patients with and without hyperlipidemia (Figure 3E).

Relationship Between Plasma Adenosine Concentration and LV Systolic or LV Diastolic Function

There was no relationship between the plasma adenosine concentration and LVEF (an indicator of LV systolic function) (P=0.249) (Figure 4A). When patients were divided into 2 groups based on LVEF <47% (n=11) and LVEF ≥47% (n=86), the plasma adenosine concentration was significantly higher in patients with LVEF <47% (220±101 nmol/L) than in those with LVEF ≥47% (149±110 nmol/L, P=0.027) (Figure 4B). We performed receiver-operating characteristic (ROC) curve analysis to determine the most appropriate cutoff values of LVEF for discrimination of elevated plasma adenosine concentrations. When the cutoff value of LVEF was 47%, the area under the curve (AUC=0.707) was greater than for other cutoff values (40%; AUC=0.694; 45%; AUC=0.694; 50%; AUC=0.594). Therefore, we used 47% as the cutoff value of LVEF for the discrimination of elevated plasma adenosine concentration. However, the plasma adenosine concentration did not correlate with E/e', an indicator of LV diastolic function. 

Results

Patients’ Characteristics and Drugs Used

Patients’ characteristics and drugs used are shown in Table 1. The mean age of the 97 patients was 71.4±9.4 years (male: 68, female: 29). Their diseases consisted of 74 cases of coronary artery disease (CAD; AP, OMI), 9 of CM (7 DCM, 2 HCM), and 14 of VHD. Biochemical data and drugs used are also shown in the Table 1.

Plasma Adenosine Concentration, Plasma BNP Level, and NYHA Classification

There was no difference in plasma adenosine concentrations among normal subjects (control), and the CAD, VHD, and CM groups (Figure 2A). There was no significant difference in the plasma adenosine concentration among patients with NYHA classes I, II, III and IV (Figure 2B). However, the plasma BNP levels increased with the severity of chronic HF according to NYHA class I–IV (Figure 2C). Plasma BNP levels were significantly higher in patients with NYHA class IV HF than in those with class I or II HF (Figure 2C). There was no relationship between plasma adenosine and BNP levels (Figure 2D).
Adenosine and Cardiac Function

function (Figure 4C,D). We defined the cutoff value of $E/e'$ as 8, an upper normal limit value.

Relationship Between Plasma Adenosine Concentration and LVDD

The plasma adenosine concentration did not correlate with LVDD, an indicator of LV dilation ($P=0.270$) (Figure 5A). However, when patients were divided into 2 groups based on LVDD (LVDD <50mm, n=50 and LVDD ≥50mm, n=47), the plasma adenosine concentration was significantly higher in patients with LVDD ≥50mm (183±122 nmol/L) than in those with LVDD <50mm (133±93 nmol/L, $P=0.030$) (Figure 5B). We also performed ROC curve analysis to determine the most appropriate cutoff values of LVDD for discrimination of elevated plasma adenosine concentration. When the cutoff value of LVDD was 50mm, AUC=0.615, which was greater than for other cutoff values (45mm: AUC=0.500; 55mm: AUC=0.508). Therefore, used 50mm as the cutoff value of LVDD for the discrimination of elevated plasma adenosine concentration.

Relationship Between Plasma Adenosine Concentration and IVSth or LVPWth

The plasma adenosine concentration inversely correlated with IVSth ($P=0.003$) (Figure 6A) and LVPWth ($P=0.0007$) (Figure 6B). The plasma adenosine level was significantly
Figure 6. (A) Relationship between plasma adenosine concentrations and interventricular septum thickness (IVSth). (B) Plasma adenosine concentrations in patients with IVSth <8 mm and IVSth ≥8 mm. (C) Relationship between plasma adenosine concentrations and LV posterior wall thickness (LVPWth). (D) Plasma adenosine concentrations in patients with LVPWth <8 mm and LVPWth ≥8 mm.

Table 2. Drugs Affecting Echocardiographic Parameters

|        | LVEF <47 (n=11) | LVEF ≥47 (n=86) | P value | LVDd <50 (n=50) | LVDd ≥50 (n=47) | P value |
|--------|-----------------|-----------------|---------|-----------------|-----------------|---------|
| ACEI/ARB (%) | 9 (81.8) | 49 (57.0) | 0.21 | 22 (44.0) | 37 (78.7) | 0.0005 |
| CCB (%) | 3 (27.3) | 43 (50.0) | 0.27 | 25 (25.0) | 21 (44.7) | 0.60 |
| BB (%) | 6 (54.5) | 26 (30.2) | 0.20 | 28 (20.0) | 18 (38.3) | 0.28 |
| Statins (%) | 3 (27.3) | 44 (51.2) | 0.24 | 26 (52.0) | 21 (44.4) | 0.47 |
| Insulin (%) | 1 (9.1) | 12 (14.0) | 0.98 | 7 (14.0) | 6 (12.8) | 0.91 |
| DPP4-I (%) | 3 (27.3) | 27 (31.4) | 0.95 | 14 (28.0) | 16 (34.0) | 0.52 |
| Nico (%) | 3 (27.3) | 17 (19.8) | 0.85 | 10 (20.0) | 10 (21.3) | 0.88 |

| IVSth <8 (n=14) | IVSth ≥8 (n=83) | P value | PWth <8 (n=16) | PWth ≥8 (n=81) | P value |
|-----------------|-----------------|---------|----------------|----------------|---------|
| ACEI/ARB (%) | 11 (78.6) | 48 (57.8) | 0.24 | 10 (62.5) | 49 (60.5) | 0.88 |
| CCB (%) | 5 (35.7) | 41 (49.4) | 0.51 | 4 (25.0) | 42 (51.9) | 0.09 |
| BB (%) | 9 (64.3) | 23 (27.7) | 0.017 | 10 (62.5) | 22 (27.2) | 0.006 |
| Statins (%) | 6 (42.9) | 41 (49.4) | 0.87 | 9 (56.3) | 38 (46.9) | 0.68 |
| Insulin (%) | 4 (28.6) | 9 (10.8) | 0.17 | 2 (12.5) | 11 (13.6) | 0.76 |
| DPP4-I (%) | 5 (35.7) | 25 (30.1) | 0.92 | 6 (37.5) | 24 (29.6) | 0.53 |
| Nico (%) | 1 (7.1) | 19 (22.9) | 0.32 | 4 (25.0) | 16 (19.8) | 0.89 |

Plasma adenosine concentration

| Drugs | Prescribed | Not prescribed | P value |
|-------|------------|----------------|---------|
| ACEI/ARB | 161.1±118.7 | 151.3±97.4 | 0.66 |
| BB | 147.4±114.3 | 162.1±108.9 | 0.54 |

IVSth, interventricular septum thickness; LVDd, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; PWth, posterior wall thickness. Other abbreviations as in Table 1.
higher in patients with IVSth <8 mm (220 ± 88 nmol/L) than in those with IVSth ≥8 mm (145 ± 110 nmol/L) (P = 0.015) (Figure 6C). The plasma adenosine level was significantly higher in patients with LVPWth <8 mm (220 ± 125 nmol/L) than in those with LVPWth ≥8 mm (145 ± 104 nmol/L) (P = 0.020) (Figure 6D). We defined the cutoff values of IVSth and LVPWth as 8 mm each, a lower normal limit value.

**Drugs Affecting Plasma Adenosine Concentration and Echocardiographic Parameters**

In the univariate analysis, the effects of drugs on LVEF, LVDd, IVSth and LVPWth that might be influenced by the plasma adenosine concentration were investigated (Table 2). Among these drugs, the incidence of angiotensin-converting enzyme inhibitors (ACEI)/angiotensin-receptor blockers (ARB) was significantly higher in the LVDd ≥50 mm group than in the LVDd <50 group (P = 0.0005). The incidence of β-blockers was significantly higher in the IVSth <8 group than in the IVSth ≥8 group (P = 0.017), and was significantly higher in the LVPWth <8 group than in the LVPW ≥8 group (P = 0.006). Therefore, ACEI/ARB and β-blockers might have affected LVDd, IVSth, and LVPWth. However, there was no difference in the plasma adenosine concentration in the presence or absence of ACEI/ARB and β-blockers. In the present study, dipyridamole and dilazep, which may affect plasma adenosine concentrations, were not prescribed.

**Discussion**

The present study demonstrated that plasma adenosine concentrations: (1) did not correlate with plasma BNP levels; (2) tended to be inversely correlated with LVEF, an indicator of LV systolic function, but did not correlate with E/e', an indicator of LV diastolic function; (3) tended to be positively correlated with LVDd, and were significantly higher in patients with LVDd ≥50 mm than in those with LVDd <50 mm; and (4) were inversely correlated with IVSth (P = 0.003) and LVPWth (P = 0.0007). Plasma adenosine concentrations were significantly higher in patients with IVSth <8 mm than in those with IVSth ≥8 mm (P = 0.015), and were significantly higher in patients with LVPWth <8 mm than in those with LVPWth ≥8 mm (P = 0.020).

It is widely accepted that adenosine has cardioprotective effects. It has been reported that adenosine release is accelerated by myocardial ischemia. In the present study, however, there were no significant differences in plasma adenosine concentrations among patients with CAD, VHD, and CM. There have been very few reports on plasma adenosine concentrations in the peripheral blood. There is only one report demonstrating that plasma adenosine concentrations increase in patients with chronic HF and that they correlated with the severity of chronic HF. In the present study, plasma adenosine concentrations did not correlate with the severity of chronic HF, and there was no difference in plasma adenosine levels among NYHA classes I, II, III, and IV. The difference in plasma adenosine concentrations between our study and that of others may be related to differences in the method used to measure plasma adenosine concentrations. We measured the plasma adenosine concentration with the LC-MS/MS system (API 4000, AB/MDS Sciex, Framingham, MA, USA) but radioimmunoassays were used in the other studies.

On the other hand, the plasma BNP level, which is regarded as an indicator of HF, tended to increase according to the severity of chronic HF (i.e., from NYHA class I to class IV) and that in NYHA class IV was significantly higher than in NYHA class I or II. Although the plasma BNP level is regarded as a diagnostic and prognostic marker of congestive HF, there was no correlation between the plasma adenosine and BNP levels, suggesting that the plasma adenosine concentration is not a sensitive diagnostic marker of congestive HF or a prognostic marker. The plasma adenosine concentration was not affected by age, sex or the presence of hypertension, diabetes mellitus, or hyperlipidemia, suggesting that it is independent of these factors.

Plasma adenosine concentrations did not correlate with LVEF. The plasma adenosine concentration was significantly higher in patients with LVEF <47% than in those with LVEF ≥47%, suggesting that it was higher in patients with deteriorated LV systolic function. These findings suggested that the plasma adenosine concentration could be an indicator of LV systolic function, and that plasma adenosine might have affected LV function. However, there was no relationship between the plasma adenosine concentration and E/e', suggesting that the plasma adenosine concentration was not an indicator of LV diastolic function. Therefore, the plasma adenosine concentration may be an indicator of LV systolic function and may counteract LV systolic dysfunction.

The plasma adenosine concentrations did not correlate with LVDd. It was significantly higher in patients with LVDd ≥50 mm than in those with LVDd <50 mm, suggesting that it was higher in patients with LV chamber dilation. Therefore, the plasma adenosine concentration may be an indicator of LV remodeling and may counteract LV chamber dilation.

Plasma adenosine concentrations inversely correlated with IVSth, and the plasma adenosine concentration was significantly higher in patients with IVSth <8 mm than in those with IVSth ≥8 mm. The plasma adenosine concentration inversely correlated with LVPWth, and was significantly higher in patients with LVPWth <8 mm than in those with LVPWth ≥8 mm. These results suggested that a higher plasma adenosine concentration may be an indicator of thinning of the IVSth or LVPWth, and that a higher plasma adenosine level might have been counteracting the thinning of IVSth and LVPWth.

Thinning of the LV wall and dilatation of the LV in end-diastole are characteristic of LV remodeling. In animal studies, it has been reported that chronic treatment with a partial adenosine A1 receptor agonist improved LV function and LV remodeling in dogs with advanced HF. Furthermore, an adenosine A2A receptor agonist has been reported to prevent thinning of the LV wall after MI in spontaneously hypertensive rats.

This is consistent with our clinical data. In human studies, it was reported that an elevated plasma adenosine concentration caused by chronic treatment with dipyridamole improved LV function and remodeling and attenuated the severity of chronic HF. In the present study, cardiac patients with higher plasma adenosine concentrations showed a decreased LVEF, greater LVDd, and thinner LV wall, suggesting LV systolic dysfunction, LV chamber...
dilation and thinning of the LVPWth and IVSth in cardiac patients with a higher plasma adenosine concentration. These results suggested that higher plasma adenosine concentrations in cardiac patients may be related to LV systolic dysfunction and LV chamber dilation and LV wall thinning.

As shown in Table 2, among the many drugs used, ACEI/ARB may be related to LVDi, and β-blockers may be related to IVSth and LVPWth. However, neither ACEI/ARB nor β-blockers influenced the plasma adenosine concentrations.

In conclusion, plasma adenosine concentrations were elevated in cardiac patients with LV systolic dysfunction, LV chamber dilation, and a thinner LV wall and IVS. Endogenous plasma adenosine may counteract the LV dysfunction, LV chamber dilation and LV wall thinning in patients with heart disease.

Novel Issues of the Present Study and Study Limitations

The novelty and advantage of our study over previous studies were: (1) we measured the plasma adenosine concentration in cardiac patients for the first time using the ESI-MS/MS method combined with HILIC separation after simple pretreatment consisting of deproteinization and ultrafiltration, and (2) we identified the possible role of endogenous adenosine in counteracting LV dysfunction, LV chamber dilation and LV wall thinning in cardiac patients.

However, there were several limitations. First, it was difficult to demonstrate that plasma adenosine was actually contributing to the improvements in LV function, LV chamber dilation and LV wall thinning in patients with HF in the setting of clinical studies. Second, we did not clarify the origin of the plasma adenosine. Although it has been reported that the plasma adenosine level is higher in plasma obtained from the brachial vein (peripheral) than been reported that the plasma adenosine level is higher in HF in the setting of clinical studies. Third, we did not clarify the origin of the plasma adenosine. Although it has been reported that the plasma adenosine level is higher in plasma obtained from the brachial vein (peripheral) than in that obtained from the coronary sinus in patients with chronic HF, suggesting that adenosine release occurs at a peripheral level and not at the myocardial level. Third, the number of cardiac patients examined was relatively small; in particular, there were few severe cases of NYHA class III or IV in the present study. A clinical study with a larger number of cardiac patients is required.

Author Contributions

Shinya M. designed the experiment, Y.K., T.T., T.A., M.H., Shingo M., M.I., Y.Y., T.N., M.K., K.N., and B.U. obtained data, and Y.K. and Shinya M. wrote the manuscript.

Acknowledgments

We thank Miss Akiko Tsujimoto for technical assistance.

Source of Funding

This study was supported by funding from Gifu University Graduate School of Medicine.

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