Pericardial Fluid Adenosine in Ischemic and Valvular Heart Disease*

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Adenosine released by ischemic myocardial cells stimulates coronary artery vasodilation. Measurement of adenosine concentrations in pericardial fluid in animal models of myocardial ischemia has been used to study the process of adenosine release. To determine whether pericardial fluid adenosine concentrations are increased in human ischemic heart disease, adenosine concentrations were measured in pericardial fluid in 23 subjects undergoing open-heart surgery for coronary artery disease. The results were compared with adenosine concentrations measured in pericardial fluid obtained from 20 subjects undergoing surgery for valvular heart disease. Adenosine concentrations also were measured in pleural fluid obtained during internal mammary artery bypass grafting. Adenosine concentrations were significantly increased in subjects with coronary artery disease compared with fluid obtained from subjects with valvular heart disease (2.47±0.24 vs 1.36±0.21 [SEM] μM [p=0.0013]). Adenosine concentrations were higher in pleural fluid than pericardial fluid from the same individuals. Adenosine concentrations were significantly correlated with pericardial fluid cell counts and lactate dehydrogenase concentrations (r=0.48; p=0.0012 and r=0.77, p=0.0001, respectively). The results are consistent with myocardial release of adenosine in ischemic heart disease. If adenosine concentrations in pericardial fluid approximate those in myocardial interstitial fluid, sufficient adenosine is present to stimulate adenosine receptor activation in coronary artery smooth muscle. (Chest 1995; 107:346-51)

LDH = lactate dehydrogenase

Key words: adenosine; myocardial ischemia; pericardial fluid

Animal studies have established that myocardial ischemia or hypoxia stimulates the release of adenosine by myocardial cells. Adenosine acts as a coronary artery vasodilator to increase the blood supply to ischemic myocardium. Attempts to validate the relevance of adenosine as a protective substance in human ischemic heart disease have proved difficult owing to the inaccessibility of human tissue for study. Previous efforts in this area have focused on measuring adenosine in coronary sinus blood. Feldman and co-workers recently used atrial pacing to induce an increase in coronary sinus adenosine in subjects with coronary artery disease. No change in coronary sinus adenosine occurred with atrial pacing in control subjects with normal coronary arteries. Unfortunately, the measurement of adenosine release is difficult owing to the low concentration of adenosine in blood and its relatively short half-life.

An increase in cardiac interstitial fluid adenosine in an animal model of myocardial ischemia has been measured in fluid collected in an epicardial well. Pericardial fluid is believed to be formed from cardiac interstitial fluid which migrates through the epicardial surface of the heart. This fluid is removed by the pericardial membrane. To our knowledge, there are no studies which have measured adenosine concentrations in human pericardial fluid. Our objective was to measure pericardial fluid adenosine, and to determine whether adenosine concentrations in subjects with ischemic heart disease differed from subjects with valvular heart disease.

MATERIALS AND METHODS

Subjects were selected from adult subjects undergoing cardiac surgery for either coronary artery disease or valvular heart disease at the Pitt County Memorial Hospital. The study was approved by the Institutional Review Committees of the Pitt County Memorial Hospital and the East Carolina University School of Medicine. Subjects were randomly selected by the principal investigator without prior knowledge of associated medical conditions or extent of coronary artery disease. An attempt was made to select equal numbers of male and female subjects with coronary artery disease and valvular heart disease. Because of this, our study population did not reflect the overall population of cardiac surgery subjects at our institution, most of whom are male patients with coronary artery disease. Subjects with combined coronary artery disease and valve disease were not studied. All subjects with valve disease had negligible coronary artery disease on heart catheterization. All subjects in both patient groups were prepared for surgery in an identical fashion, and the initial surgical procedure was similar in both groups. Subjects were premedicated for...
surgery with either intravenous midazolam or lorazepam. Intravenously administered cefuroxime was given prior to surgery for prevention of wound infection. Anesthesia was maintained with fentanyl or sufentanil citrate. Paralysis was induced and maintained with either pancuronium or vecuronium. Supplemental oxygen was administered during the procedure sufficient to maintain the hemoglobin saturation at 100% as measured by pulse oximetry and arterial blood gas monitoring. At no time were any of the subjects hypoxic. The chest was opened by median sternotomy. Pleural fluid was collected in ten subjects undergoing left internal mammary artery grafting. The fluid was collected prior to graft harvesting using a suction catheter. Pericardial fluid was collected with a suction catheter inserted into the pericardial sac immediately after the pericardial sac was opened. Following collection, the fluid was immediately chilled on ice. To inhibit the formation or degradation of adenosine, or both, solutions of 3 μM 9-erythro-2-(hydroxy-non-3yl) adenosine, 26 μM dipyridamole, and 6 mM α, β-methylene adenosine 5'-diphosphate dissolved in saline solution were immediately added. The final concentrations of inhibitors were 0.25, 2, and 60 μM, respectively. Only samples that were free of blood contamination during the surgical procedure were collected. Following collection of the fluid, cells were counted on a hemocytometer and the cellular component was separated by centrifugation (400g for 8 min). The cellular pellet was resuspended in Hank's balanced salt solution and prepared for cytologic examination and differential counting by centrifugation (Cytospin, Southern Instruments, Sewickley, Pa), followed by staining with Diff-Quick method (Baxter Scientific, McGaw Park, Ill). Macrophages and mesothelial cells could not be reliably distinguished and were treated as a group. The pericardial fluid supernatant was centrifuged a second time (38,000g for 15 min). Aliquots of the lavage fluid were frozen at −70°C for subsequent analysis.

Samples were prepared for measurement of adenosine by adding sufficient 50% trichloracetic acid to obtain a final concentration of 10% trichloracetic acid. The solution was vortexed and chilled at 4°C for 10 min. The solution was centrifuged at 10,000g in a microcentrifuge for 10 min. The supernatant was removed and washed to remove excess trichloracetic acid by extraction (4 volumes) with water-saturated diethyl ether. The solution was lyophilized and resuspended in one half of the original volume with saline solution.

Adenosine was measured in 50 μL of the fluid using a Beckman high-performance liquid chromatography system with a Waters ultraviolet light detector set at a wavelength of 254 nm. The sample was injected into a 3.9 mm×15 cm NOVA-pak C18 reversed phase column (Waters Instruments, Rochester, Minn) and eluted with a mobile phase consisting of 4 mM KH2PO4, and 8% methanol (volume per volume), pH 5.0, at a flow rate of 1.0 mL/min. The presence or absence of adenosine was confirmed by the addition of a known amount of adenosine to augment adenosine peaks and by the technique of enzymatic shift wherein adenosine deaminase is used to convert adenosine to inosine. In this assay system, adenosine appeared as a single distinct peak with a retention time of about 8.5 to 9 min. Adenosine concentrations were calculated by integrating the area under the curve and comparing this with adenosine standards of known concentration. The assay was capable of detecting adenosine at concentrations as low as 100 nmol, which is the detection limit for ultraviolet-absorbing nucleosides.

Protein concentration in the lavage fluid was measured using a standard Lowry assay. Lactate dehydrogenase (LDH) activity was measured by a colorimetric method using a reagent test system (Sigma Diagnostics, St. Louis). Nonmetabolized pyruvate substrate was measured in a spectrophotometer set at a wavelength of 440 nm.

Pericardial fluid was prepared for measurement of adenosine deaminase activity by dialyzing 0.5 ml for 20 hrs using an analyzer (Spectra/Per, Spectrum Laboratories, Los Angeles), M.W. cutoff 3,500. This was done to remove the deaminase inhibitor. Adenosine deaminase activity (units per liter at 37°C) was determined colorimetrically by the reaction of NH4+ with phenol nitroprusside in an alkaline solution. Absorbance was measured at 625 nm.

Analysis of variance was used in the analysis of the adenosine data (SAS Computer Software Systems, Cary, NC). We investigated whether sex or age might explain differences observed between patient groups. Distributions were assessed for normality using the Shapiro-Wilk test. Data were normalized using logarithmic transformation when appropriate. The relationship of adenosine concentrations with LDH and protein concentrations and cell numbers were determined by Pearson's correlation coefficients. All results are provided as the mean ± SEM and probability values less than 0.05 were considered significant.

**RESULTS**

This study population consisted of 12 male and 11 female subjects with coronary artery disease and 10 male and 10 female subjects with valvular heart disease. The mean age of the subjects with coronary artery disease was 59.7 ± 2.2 years, and the mean age of the subjects with valvular heart disease was 60.4 ± 3.2 years. The ejection fraction, estimated at the time of cardiac catheterization, was 55 ± 3% in subjects with coronary artery disease and 52 ± 4% in subjects with valvular heart disease. Subjects with valvular heart disease did not have coronary artery disease. Cardiac catheterization was performed within 2 months of heart surgery. Subjects undergoing coronary artery bypass surgery had severe coro-

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**Table 1—Results of Pericardial Fluid Analysis***

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coronary Artery Disease</th>
<th>Valvular Heart Disease</th>
<th>Probability Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine, μM</td>
<td>2.47 ± 0.24</td>
<td>1.36 ± 0.21</td>
<td>0.0013</td>
</tr>
<tr>
<td>LDH, IU</td>
<td>395 ± 45</td>
<td>302 ± 44 IU</td>
<td>0.002</td>
</tr>
<tr>
<td>Protein, g%</td>
<td>2.77 ± 0.16</td>
<td>2.55 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Cells/mL×10⁶ Cells</td>
<td>2.65 ± 0.32</td>
<td>1.97 ± 0.31</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>20.0 ± 4.3</td>
<td>20.0 ± 4.4</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>1.9 ± 1.0</td>
<td>6.1 ± 4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Macrophage/mesothelial cell, %</td>
<td>78.1 ± 5.1</td>
<td>74.2 ± 6.0</td>
<td>NS</td>
</tr>
<tr>
<td>Volume, mL</td>
<td>6.5 ± 0.67</td>
<td>12.9 ± 2.0</td>
<td>0.009</td>
</tr>
</tbody>
</table>

*All data are expressed as mean ± SEM.
monary artery disease. Thirteen subjects had a history of myocardial infarction. Seven of these had recent infarctions (within 4 days prior to surgery). Four subjects had stenosis of the left main coronary artery. Ten subjects had stenosis of three vessels, six subjects had two-vessel disease, and three subjects had single-vessel disease. The indication for bypass surgery in all cases was unstable angina. Twelve subjects had chest pain at rest.

The results of pericardial fluid analysis are summarized in Table 1. The distribution of individual measurements of adenosine in both subject groups is illustrated in Figure 1. Adenosine concentrations were significantly increased in subjects with coronary artery disease. Pericardial fluid LDH was significantly increased in subjects with coronary artery disease, but the mean serum LDH was actually slightly lower in this group (193 ± 10 vs 220 ± 11 IU for control subjects). The volume of pericardial fluid collected was significantly higher (p=0.009) in subjects with valvular heart disease than coronary artery disease, while the cellularity of the fluid, expressed as cells per milliliter of fluid collected, was higher in the group with coronary artery disease.

An attempt was made to determine whether adenosine concentrations were correlated with the cellularity of the fluid or other parameters. Significant correlations were found between adenosine and pericardial fluid LDH (r=0.77; p=0.0001), and adenosine and cells per milliliter (r=0.48, p=0.0012). These results are illustrated in Figures 2 and 3. The cellular composition of the pleural fluid of subjects with valve disease and coronary artery disease was remarkably similar, with a large majority of cells being either macrophages or mesothelial cells. Red blood cell numbers were minimal in most cases. In the nine subjects with the most RBCs, concentrations of RBCs ranged from 10^4/mL to 2.3×10^5/mL. Adenosine concentrations did not correlate with the protein content of the fluid (r=0.27; p=0.08). No significant age or sex differences were observed.

An attempt was made to correlate adenosine concentrations in pericardial fluid with the severity of coronary artery disease found at angiography and with the presence of myocardial infarction. Seven subjects had a recent myocardial infarction (within 4 days of surgery) indicated by elevation of serum creatine kinase and LDH isoenzymes. The mean pericardial fluid adenosine concentration in these

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21708/)  
**Figure 1.** Distribution of adenosine concentrations in pericardial fluid in subjects with coronary artery disease and valvular heart disease. The horizontal lines indicate the mean of group.

![Figure 2](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21708/)  
**Figure 2.** Correlation of pericardial fluid adenosine concentrations and LDH activity. The data points represent values obtained in all study subjects. The micromolar concentrations of adenosine are expressed on the abscissa, and the units of LDH activity per liter are expressed on the ordinate.

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21708/)  
**Figure 3.** Correlation of pericardial fluid adenosine concentrations and cellularity. The data represent values obtained in all study subjects. The micromolar concentrations of adenosine are expressed on the abscissa and the cellularity of the fluid in cells per milliliter is expressed on the ordinate.
subjects was 2.01 ± 0.39 compared with a mean adenosine concentration of 2.95 ± 0.42 in the other 16 subjects without myocardial infarction. Four subjects had left main coronary artery disease. The mean adenosine concentration in pericardial fluid of these subjects was 2.86 ± 0.47 μM. (Two of these subjects also had acute myocardial infarction.) Adenosine concentrations also were similar in subjects with chest pain at rest and in those subjects with pain associated with exercise.

An attempt was made to determine whether differences in adenosine concentrations in pericardial fluid might be explained by differences in adenosine deaminase activity. Mean adenosine deaminase activity was 10.1 ± 1.0 U/L in a subset of 10 subjects with coronary artery disease and 9.8 ± 1.0 U/L in 10 subjects with valvular heart disease.

The stability of adenosine in the presence of 9-erythro-2-(hydroxy-non-3yl) adenine; α, β-methylene adenosine 5’-diphosphate, and dipyridamole was tested in pericardial fluid obtained from four additional subjects with coronary artery disease. Adenosine was added to the pericardial fluid containing the inhibitors to ensure concentrations of at least 1 μM. Adenosine concentrations remained stable (within 15% of baseline) for at least 20 min when incubated at 37°C.

Adenosine concentrations were measured in the pleural fluid of 10 subjects with coronary artery disease. The mean pleural fluid adenosine concentration was 9.45 ± 2.88 μM. The mean adenosine concentration in pericardial fluid of the same subjects was 2.94 ± 0.44 μM. Adenosine deaminase activity was measured in the pleural fluid of seven subjects and was found to average 9.2 ± 1.8 U/L.

**DISCUSSION**

The results of our study are consistent with the hypothesis that adenosine is released by ischemic human myocardium. Adenosine concentrations were approximately twofold higher in pericardial fluid obtained from subjects with coronary artery disease than that obtained from a control group with valvular heart disease and normal coronary arteries. Previous studies in humans have had difficulty in measuring adenosine release from the myocardium owing to the very rapid metabolism of adenosine in blood. In a recent study of venous effluent coronary sinus blood, adenosine concentrations increased from 0.132 to 0.265 μM during cardiac pacing-induced tachycardia in subjects with coronary artery disease.7 No increase in coronary sinus blood adenosine was measured in subjects without coronary artery disease. To our knowledge, no previous studies have measured adenosine concentrations in human pericardial fluid. The concentrations measured in pericardial fluid in our study are remarkably high, considering that concentrations of adenosine in plasma have been reported to be about 0.05 μM.10 High concentrations of adenosine in pericardial fluid might have resulted from adenosine release during myocardial ischemia prior to or during surgery. While we have no direct evidence that myocardial ischemia was present in our subjects at the time of surgery, all patients undergoing bypass surgery had severe coronary artery disease. Most of these subjects had three-vessel disease with high-grade stenotic lesions. Half the subjects had resting pain and some very likely had silent ischemia. Unfortunately, there is no accepted biochemical marker for ongoing myocardial ischemia which is available for comparison with adenosine. The induction of transient ischemia during surgery would be required to prove a direct link between ischemia and adenosine release into pericardial fluid. This was beyond the scope of our study.

Adenosine concentrations greater than 1 μM have produced human coronary artery vasodilation in vitro.18,19 Accordingly, the concentrations of adenosine measured in pericardial fluid in valvular heart disease are at the threshold for producing coronary artery vasodilation. Higher concentrations such as were measured in many of our subjects with coronary artery disease would be capable of activating aortic second sound receptors on coronary artery smooth muscle, if adenosine concentrations in pericardial fluid approximate those in myocardial interstitial fluid.

Measurement of adenosine in pericardial fluid formed from cardiac interstitial fluid has been used to study the release of adenosine in animal models of myocardial ischemia.11,12 Studies which have measured pericardial fluid adenosine in animals have assumed that adenosine is released from the myocardium and migrates to the epicardial surface where it mixes with pericardial fluid. This fluid is later absorbed by the pericardium. The use of an epicardial well to collect fluid migrating from the myocardium has allowed investigators to estimate adenosine concentrations in cardiac interstitial fluid. Adenosine concentrations in our study were higher than that measured in epicardial wells in nonischemic dog hearts (0.55 μM).12

Several alternative explanations may account for the relatively high pericardial fluid adenosine concentrations observed in subjects with coronary artery disease. The association of adenosine concentrations with the cellularity and LDH activity of the pericardial fluid raises the possibility that adenosine may appear in the fluid as a result of changes in cell membrane permeability and leakage of cytoplasmic fluid. Although pericardial fluid was collected in a manner so as to minimize trauma to the pericardium...
and epicardium, cells residing in the pericardial fluid may have been damaged during pericardial fluid collection. Adenosine and LDH, an intracellular enzyme, may thus be markers of cell injury or cell membrane disruption. The association of adenosine concentrations and cellularity of fluid was observed in a study of adenosine in bronchoalveolar lavage fluid. Evidence against this explanation is the lack of correlation between adenosine and protein concentrations. Cell membrane damage would be expected to result in leakage of cytoplasmic proteins as well as LDH and adenosine. Subjects with coronary artery disease were more likely to have received medication before cardiac surgery. In particular, nitrates, β-adrenergic antagonists, heparin, and calcium-channel antagonists were prescribed more frequently in subjects with coronary artery disease. Subjects with valvular heart disease were treated more frequently with digoxin, furosemide, and angiotensin-converting enzyme inhibitors. Oral medications were withheld the night prior to surgery. We cannot exclude a role for medication as a possible cause for the increased adenosine concentrations measured in the pericardial fluid of subjects with coronary artery disease. Finally, it is possible that lower adenosine concentrations observed in the patients with valvular heart disease may reflect dilution factors associated with increased formation of cardiac interstitial fluid. The lower cellularity and the lower LDH concentrations also are consistent with this possibility, but protein concentrations were nearly equal in both groups. The similarity of ejection fractions in each group suggests that poor left ventricular function was not responsible for differences in pericardial fluid volumes.

High concentrations of adenosine in pericardial fluid could be perpetuated by a relative lack of adenosine deaminase in the fluid compared with plasma. Adenosine deaminase activity in pericardial fluid, however, was similar to the enzyme activity reported in serum. Adenosine deaminase activity was nearly identical in the pericardial fluid of subjects with coronary artery disease and valvular heart disease.

The availability of sampling pleural fluid during internal mammary artery bypass grafting provided a unique opportunity to measure adenosine in pleural fluid. Ordinarily, pleural fluid in normal individuals is inaccessible owing to the very small amount present. Adenosine provokes bronchoconstriction in subjects with asthma, and micromolar concentrations of adenosine have been measured in bronchoalveolar lavage fluid in asthmatic subjects. Surprisingly high concentrations of adenosine also were measured in pleural fluid. In fact, adenosine concentrations were actually higher in pleural fluid than in pericardial fluid in the same subjects with coronary artery disease. Since pleural fluid may be formed from lung interstitial fluid, micromolar adenosine concentrations also may be present in lung interstitial fluid. Adenosine would thus be present in sufficient concentration to provoke bronchoconstriction in asthmatic subjects by stimulating either mast cell release of mediators or by directly stimulating bronchial smooth muscle contraction. The mean adenosine deaminase activity of 9.2 U/L measured in the pleural fluid of seven subjects was very similar to that recently reported in 88 transudative pleural effusions by Valdés and coworkers. The majority (78%) of these transudative effusions were associated with congestive heart failure.

In summary, the results obtained in this study are consistent with the hypothesis that adenosine is released by ischemic myocardium. Adenosine is present in sufficiently high concentrations in pericardial fluid to activate receptors which stimulate coronary artery vasodilation.

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