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E. BORENTE
Certifying Officer
SELECTIVE VASODILATION BY CONTINUOUS ADENOSINE INFUSION

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Filed: Mar. 15, 1993

Related U.S. Application Data


Field of Search

References Cited

U.S. PATENT DOCUMENTS

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Primary Examiner—John Kight
Assistant Examiner—L. Eric Crane
Attorney, Agent, or Firm—White & Case

ABSTRACT

This invention is concerned with the use of adenosine as an agent for the treatment of human beings. More particularly, this invention is concerned with the administration of adenosine to human patients by continuous intravenous infusion for, inter alia, control of blood pressure, use as a selective vasodilator, decreasing pulmonary vascular resistance, treating acute pulmonary hypertension in conjunction with idiopathic respiratory distress syndrome, in diagnosing pulmonary hypertension in conjunction with cardiac septum defects, in percutaneous transluminal angioplasty (PTCA), in coronary thrombolysis (CTI) and in radionucleide scintigraphy.
Accordingly, for adenosine to be of practical value for use as a vasodilator, it must be administered continuously to maintain plasma levels sufficiently high to achieve vasodilation. The problem, however, is that such continuous administration could lead to undesired side effects, such as the above-noted heart blockage.

It also should be noted that compounds commonly used as vasodilators, such as sodium nitroprusside, nitroglycerine, isoflurane, hydralazine, prazosin and the like, have various side effects. For example, sodium nitroprusside has the drawbacks of tachyphylaxis and rebound hypertension, apparently caused by autogenous generation of angiotensin to counteract the hypotensive effect of the nitroprusside. As a consequence, the dosage of nitroprusside must be progressively increased with continued use to overcome the hypertensive effect of angiotensin, and there is a risk of rebound due to the presence of residual excess angiotensin. Nitroglycerine and prazosin suffer from the drawbacks of slow onset and unpredictable action. Isoflurane and sodium nitroprusside both have a tendency to reduce cardiac blood flow, while nitroprusside, hydralazine and prazosin increase heart rate.

Accordingly, there remains a need for a vasodilator suitable for administration by continuous intravenous infusion.

The present invention is based upon the discovery that adenosine can be administered to human patients under conditions such that significant vasodilation is achieved without the occurrence of significant heart blockage. It is based on the further discovery that adenosine has a unique, and heretofore unappreciated, activity profile in humans which differs significantly from the profiles of heretofore commonly used vasodilators. As a consequence of this discovery, it has been discovered that adenosine may be employed for the treatment of a variety of conditions by continuous intravenous infusion techniques. In particular, and as will be illustrated in greater detail below, adenosine has been found to have the following characteristics:

1. It has selective vasodilating activity, in that its effect is limited to a cardiac after-load effect. That is, its activity is limited to dilatation of arteries and it has little or no effect on cardiac pre-load, i.e., as a dilator of veins.

2. Although adenosine has significant action in blocking atrio-ventricular (A-V) conductance by bolus injection, it can be administered by continuous infusion and have significant useful vasodilating action at dosages below those at which it has significant A-V activity.

3. Adenosine has significant hypotensive activity without the occurrence of significant tachyphylaxis, apparently because adenosine blocks the renin-angiotensin system of the kidney, thus preventing hypertension due to the formation of angiotensin in response to hypotension.

4. Adenosine’s effect is readily controlled because it is active at relatively small doses and because of its short plasma half-life (10-20 seconds). In addition, its activity quickly ceases when adenosine administration is terminated.

5. Adenosine is capable of significantly increasing cardiac output without significantly increasing cardiac work.

6. Atenosin, in the amounts used in accordance with the invention, is essentially non-toxic. It is rapidly taken up by the body to form ATP, and upon degradation its metabolites are present at or below levels normally resulting from physical exercise.

The foregoing activity profile permits continuous infusion of adenosine for controlled hypotension during surgery, for control of various forms of hypertensive crisis, to improve coronary circulation during surgery in patients with
ischemic heart disease, for reducing the incidence of coronary graft occlusion by increasing graft flow following coronary bypass surgery, and for reducing platelet loss during cardiac bypass surgery. It has also been found that adenosine may be used for decreasing pulmonary vascular resistance, for treating acute pulmonary hypertension, for diagnosing the operability of the pulmonary vasculature in patients with pulmonary hypertension in conjunction with cardiac septum defects. Adenosine also is useful in inhibiting clot formation during percutaneous transluminal coronary angioplasty (PTCA) and coronary thrombolysis (CTI), as well as an aid in visualizing miocardial ischemia for radionuclide scintigraphy.

In accordance with this invention, adenosine may be administered to human patients by continuous intravenous infusion to provide significant vasodilation and without significant heart blockage under two conditions. First, the heart blocking action of adenosine is not detected during general anesthesia when the rate of administration is 0.35 milligrams per kilogram per minute or less. Second, the heart blocking action of adenosine is not detected even in conscious patients, at rates of administration of about 0.10 milligram per kilogram per minute or less.

For purposes of this invention, adenosine can be administered to the patient in any pharmaceutically acceptable form for use in continuous, intravenous infusion. A preferred form is an aqueous solution of adenosine, and more preferably adenosine in isotonic saline. The concentration of adenosine in the solution is not narrowly critical, although concentrations of at least about 5 millimol (about 1.5 milligrams per milliliter) of solution are desired to avoid the need for excessive infusion rates to achieve desired serum levels. When administering adenosine to small children, however it is possible to use concentrations as low as about 0.1 mg/ml. The concentration may be as high as the solubility limit of adenosine (about 20 millimols per liter or 5.5 to 6 milligrams per milliliter), if desired.

With the use of continuous intravenous infusion in accordance with this invention, the unit dosage form typically has a volume of at least 250 milliliters, and preferably in the range of 250 to 500 milliliters, to provide an adequate supply of adenosine. Consequently, the unit dosage form generally will contain from about 0.4 to about 3 grams of adenosine, although much smaller units are effective.

For purposes of this invention, adenosine can be administered to patients in any pharmacologically acceptable form for use in continuous, intravenous infusion. A preferred form is an aqueous solution of adenosine, and more preferably adenosine in isotonic saline. The concentration of adenosine in the solution is not narrowly critical, although concentrations of at least about 5 millimol (about 1.5 milligrams per milliliter) of solution are desired to avoid the need for excessive infusion rates to achieve desired serum levels. When administering adenosine to small children, however it is possible to use concentrations as low as about 0.1 mg/ml. The concentration may be as high as the solubility limit of adenosine (about 20 millimols per liter or 5.5 to 6 milligrams per milliliter), if desired.

When used for continuous infusion in accordance with this invention, the unit dosage form typically has a volume of at least 250 milliliters, and preferably in the range of 250 to 500 milliliters, to provide an adequate supply of adenosine. Consequently, the unit dosage form generally will contain from about 0.4 to about 3 grams of adenosine. In small children, the unit dose will, of course, be correspondingly smaller than it is for adults.

The adenosine solution should be sterile and free from fungi and bacteria. Such solutions have been found to be stable at room temperature for at least two years. Such solutions are prepared by mixing adenosine with the aqueous carrier, e.g., water or an isotonic solution, and other desired ingredients, to achieve a solution having the desired concentration, and thereafter sterilizing the solution.

Continuous infusion can be performed using any technique known to the art. Because adenosine has such a short plasma half-life and it is active at relatively low concentrations, it is desired that the method be one which minimizes or avoids fluctuations of serum adenosine levels. Accordingly use of high precision roller pumps is preferred.

As is noted above, the present invention has numerous specific applications, depending upon adenosine dosage levels and whether or not adenosine is administered to anesthetized or conscious patients. The first general category of applications is that in which adenosine is continuously administered to a patient undergoing surgery under general anesthesia at doses that do not induce heart block. Specific applications include controlled hypotension during surgery, in particular dissection and clipping of cerebral arterial aneurysms; control of hypertension crisis during surgery, for example due to release of catecholamines in the course of pheochromocytoma surgery; and improved coronary circulation and after-load reduction during abdominal aortic aneurysm surgery, especially in patients with ischemic heart disease. For such uses, dosage rates of the order of 0.05 to about 0.3 milligrams per kilogram of body weight per minute are effective amounts.

The second general category of continuous adenosine infusion applications is that in which adenosine is administered to conscious patients, also at levels below which adenosine exhibits significant heart blocking action. These levels are typically achieved at administration rates of 0.05 milligram of adenosine per kilogram per minute or less. Specific examples of conditions which may be treated with adenosine in conscious patients include prevention of occlusion of cardiac bypass grafts following bypass surgery, increase cardiac output in patients with low cardiac output, and use of adenosine as an adjunct to dopaminc treatment for shock.

Blood levels of adenosine which result from an administration rate of about 10–30 micrograms per kilogram of body weight per minute (0.010–0.030 mg. per kg. per minute) amount can be used in a number of additional ways, e.g., to decrease pulmonary vascular resistance, to treat acute pulmonary hypertension and acute pulmonary hypertension in conjunction with idiopathic respiratory distress syndrome (IRDS), and to diagnose pulmonary hypertension in conjunction with cardiac septum defects.

The following examples of continuous intravenous infusion of adenosine in accordance with this invention.

EXAMPLE I

CONTROLLED HYPOTENSION DURING ANESTHESIA

It is frequently desired to reduce the blood pressure of patients during surgery. For example, in the case of dissection and clipping of cerebral arterial aneurysms, controlled hypotension is desired to reduce the aneurysm wall tension in order to minimize the risk of rupture and bleeding. Controlled hypotension is also used to reduce bleeding during other forms of surgery.

Prior to this invention, vasodilators, such as sodium nitroprusside and nitroglycerine, were used for this purpose, but both have drawbacks. For example, sodium nitroprusside suffers from tachyphylaxis, or the need to increase the dose of adenosine with time due to the release of angiotensin. In addition, rebound hypertension also has been observed following use of nitroprusside. Nitroglycerine is characterized by a slow onset of action and unpredictable action.

Adenosine has been found to be a remarkably effective agent for inducing controlled hypotension during surgery. Adenosine, when administered in effective amounts, has a very rapid hypotensive effect which can be rapidly terminated due to its short half-life. Moreover, adenosine does not cause tachyphylaxis, apparently because it blocks the renin-angiotensin system of the kidney, thereby preventing formation of angiotensin which tends to counteract the hypotension. For the same reason, rebound hypertension is avoided after discontinuation of infusion.

For this indication, adenosine typically is administered intravenously via the left basilic vein or via a central vein in
an amount (or at a rate) sufficient to achieve the desired hypotensive effect. It has been found that lowering of mean arterial blood pressure to as low as 40 millimeters of mercury, as measured by a cannula in the left radial artery, is readily achieved without significant side effects. In particular, so long as the patient is under anesthesia, no blockage of atrio-ventricular conduction is observed.

The actual plasma levels of adenosine employed for controlled hypotension will vary, depending upon such factors as the particular patient, the age of the patient and the desired degree of hypotension. As a general rule, however, a reduction of mean arterial blood pressure to 40 to 50 mm Hg is achieved by administration of adenosine at a rate of from about 0.2 to about 0.35 milligrams of adenosine per kilogram of body weight per minute. The amount of adenosine required to achieve a given degree of hypotension can be reduced if adenosine uptake inhibitors, such as dipyridamole, are also administered to the patient. The possibility that adenosine might be useful for inducing controlled hypotension in humans was suggested by Kassel et al., J. Neurosurg., 58: 69-76 (1983), based upon tests in dogs. However, this study was performed during the administration of dipyridamole, another vasodilator that potentiates the effect of adenosine by inhibiting cellular uptake of adenosine. The dose of dipyridamole (1 mg/kg) was high, and it in fact induced a 20% reduction of the mean arterial blood pressure. The hypotensive effect of adenosine was then studied upon this hypotensive dose of dipyridamole. It was reported that hypotension to a mean arterial pressure of 40 mm Hg could be induced and maintained with an infusion of 0.4 gram of adenosine per 100 milliliters of normal saline, at a dose of 50 μg/kg/minute. When dipyridamole was excluded in a pilot study, as much as 5-10 mg/kg/minute was required for the induction of hypotension, thereby creating an excessive fluid load. Kassel et al. noted that induction of hypotension in dogs is difficult, and speculated that "adenosine alone, without the potentiating effects of dipyridamole, may be sufficient to produce hypotension in man without excessive volumes of fluid". As noted above, effective induction of hypotension in man is achieved at adenosine dose levels of only 0.2 to 0.35 mg/kg/minute, or 30 to 50 times lower levels than effective levels in the dog. Such low rates are hardly predictable from the information of Kassel et al.

In a study intended to demonstrate use of continuous infusion of adenosine to effect controlled hypotension in man without patients with no known cardiovascular diseases (seven men and three women, ages 35-58 years), scheduled for intracranial aneurysm surgery, were selected. One hour before the operation the patients were premedicated orally with diazepam (10-20 mg) and hydroxyzine (0.5 mg) and droperidol (0.1 mg/kg) were given intravenously before induction of anesthesia. Induction was started with thiopental (5 mg/kg) followed by phenerganide (1-2 mg), a synthetic opiate with pharmacodynamic resembling fentanyl but with a longer duration of action and 1% of its analgesic potency.

Pancuronium bromide (0.1 mg/kg) was given to facilitate endotracheal intubation. Anesthesia was maintained by supplementary doses of phenerganide and droperidol, as required. The total dose of droperidol did not exceed 0.2 mg/kg, and was administered within the first 2 hours of anesthesia. Phenerganide was supplemented regularly to prevent the blood pressure from exceeding the preanesthetic level (approx. 1 mg/kg/30-60 min). Controlled hyperventilation was employed with a humidified gas mixture of 60% N2O in oxygen to maintain PaCO2 values at approximately 30 mmHg (±1.5 SSM). Mannitol (1-1.5 g per kg) was given routinely at the start of the operation (e.g., 1-2 hours prior to the controlled hypotension). The patients were operated on in the horizontal lapar position.

A 1.2-mm plastic cannula was introduced into the left radial artery to monitor systemic arterial blood pressure (MAPB) and collect arterial blood. A balloon-tipped, flow-directed, quadricipital Swan-Ganz catheter (Model 95A-311-7.5 F, BIOPHYS) was inserted percutaneously via the left basilic vein, and its correct position in the pulmonary artery was determined by pressure tracings. The catheter was used for the monitoring of mean right atrial pressure (RAP), mean pulmonary artery pressure (PAP), and mean pulmonary arterial wedge pressure (PCW). The determination of cardiac output and collection of mixed venous blood and for the infusion of adenosine. Another plastic cannula was introduced percutaneously, in a retrograde direction, into the right jugular bulb for the collection of blood. The correct position was verified by x-ray.

The ECG was monitored with a standard chest (V5) lead. Heart rate was determined from the R-R interval. Blood pressures were measured by transducers placed at the midthoracic level. Cardiac output (QT) was determined in triplicate according to the thermodilution technique with a Cardiac output computer (Edwards Lab, model 9510). Isotonic glucose, 10 ml at 1°C, was used as a thermal indicator. The ECG, heart rate, blood pressures, and thermodilution curves were recorded on a Grass® polygraph.

Blood gases were measured with appropriate electrodes for pH, PO2, and PO2 (Radiometer, Copenhagen). The hemoglobin concentration was determined spectrophotometrically. Samples for the determination of adenosine and its metabolites were collected as described by Solllev et al., Acta Physiol. Scand., 120: 171-76 (1984). Adenosine and its metabolites were purified and analyzed by HPLC as described by Fredholm and Sollevi, J. Physiol. (London), 313: 351-67 (1981). Hypoxanthine, xanthine, and uric acid were analyzed by HPLC according to the method of Schweinsberg and Loo, J. Chromatogr., 181: 103-7 (1980). Arterial levels of dipyridamole were determined by HPLC. J. Chromatogr., 162: 98-103 (1979). Blood lactate was measured according to Treit-Hansen and Siggaard-Andersen, Scand. Clin. Lab. Invest., 27:15-19 (1971).

Measurements and blood samplings were performed immediately before hypotension, as late as possible during hypotension (1-5 min prior to terminating the infusion) and approximately 30 min after the hypotensive period. Dipyridamole (5 mg per ml) was infused iv (0.3-0.4 mg per kg, over a period of 5-10 min) approximately 20 minutes prior to the induction of controlled hypotension. This dose of dipyridamole produced clinically relevant drug levels in the plasma (1.2±0.5 μM, SSM) during the hypotensive periods. (See Pedersen, J. Chromatogr., 162: 98-103 (1979).

Adenosine (5 mM, 1.34 mg per ml in isotonic saline) was administered by continuous infusion (Clinitron roller pump, 2102A, superior vasa cava) for 12-71 minutes (±3±3 SEM) at a rate of 0.01-0.32 mg per kg per min (±0.14±0.04 SEM, corresponding to 8.5±2.7 mg per min). The infusion was started at a rate of 0.01 mg per kg per minute, which was doubled at 15 second intervals until the desired MAPB level of 40-50 mmHg was reached. The corresponding volume of infused adenosine solution ranged from 0.5 to 17 ml per min (±6±2 SEM). The mean hypotensive period was 32±8 min. The total adenosine dose did not exceed 1.5 times. Serum catecholamines were determined before and on the two consecutive days after operation. The standard ECG was recorded the day before and the day after operation.
Systemic vascular resistance (SVR) was derived from the formula

\[
SVR(\text{mmHg per liter min}) = \frac{MABP - PAP}{QT}
\]

and pulmonary vascular resistance (PVR) from the formula

\[
PVR = \frac{PAP - PCWP}{QT}
\]

Oxygen content was derived from the formula \(SO_2 \times \frac{1.34 \times Hb \times P_O_2 + 0.03}{100} \) (Fock et al., Br. J. Anesth., 42: 803-4 (1970). The arteriovenous oxygen content difference (AVDO₂) was determined and used to calculate total oxygen consumption (VO₂) as the product of AVDO₂ and QT.

*SO₂* = Oxygen saturation. The results of this work are summarized in Tables I-IV, in which data are presented as means ±SEM. The statistical significance (control 1 vs. adenosine and control 1 vs. control 2) was determined by Student’s t test for paired data. A P value of < 0.05 was regarded as significant.

The purine levels of nine of the patients were determined prior to, during and after adenosine-induced controlled hypotension. The results are summarized in Table I.

### TABLE I

<table>
<thead>
<tr>
<th>Purine Levels (μM) in Arterial Plasma Before, During, and After Adenosine-induced Controlled Hypotension in Nine Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
</tr>
<tr>
<td>Inosine</td>
</tr>
<tr>
<td>Hypoxanthine</td>
</tr>
<tr>
<td>Xanthine</td>
</tr>
<tr>
<td>Uric acid</td>
</tr>
</tbody>
</table>

*P < 0.01
**P < 0.05, denotes significantly different from control 1.
*P < 0.01

As is evident from Table I, adenosine is present in the 10⁻⁷M range during basal conditions. Continuous infusion of adenosine increased the arterial adenosine level to 2.45±0.65 μM. The adenosine metabolites inosine and hypoxanthine were increased during the infusion, whereas xanthine and uric acid levels were unaffected. Once the desired blood pressure level was reached, the infusion rate could be kept constant throughout the hypotensive period. After termination of the infusion, the arterial adenosine levels returned to control values within 3–9 min. Inosine was eliminated more slowly from the circulation and remained slightly above basal levels 20–40 min after the infusion.

The central hemodynamic variables were measured in all 10 patients before, during and 30 minutes after controlled hypotension and are summarized in Table II.

### TABLE II

<table>
<thead>
<tr>
<th>Central Hemodynamic Variables Before, During and 30 Min After Adenosine-induced Controlled Hypotension in Ten Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
</tr>
<tr>
<td>Right atrial pressure (mmHg)</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure (mmHg)</td>
</tr>
<tr>
<td>Heart rate (beats per min)</td>
</tr>
<tr>
<td>Cardiac output (liters per min)</td>
</tr>
<tr>
<td>Systemic vascular resistance (mmHg per min)</td>
</tr>
<tr>
<td>Pulmonary vascular resistance (mmHg per liter min)</td>
</tr>
</tbody>
</table>

*P < 0.01
**P < 0.05, denotes significantly different from control 1.

The infusion of dipyridamole decreased MABP by approximately 10 mmHg in five of the patients. At the start of the adenosine infusion, MABP was not significantly different from the predipyridamole level (82±3 vs. 86±3 mmHg) as shown in Table II. Adenosine induced a decrease in MABP to 46 mmHg (43±6%) within 1–2 minutes. The decrease in MABP was caused by a parallel decrease in both systolic and diastolic pressure. The MABP was stable throughout the hypotensive period. Cardiac output increased from 4.9 to 6.91 per minute (44±5%) in parallel with a small increase in heart rate of 4±2 beats per minute. The SVR decreased from 16.7 to 6.2 mmHg per liter per minute, corresponding to a decrease of 61±3%, whereas PVR was unchanged. RAP, PAP, and PCWP were not influenced by adenosine.

After discontinuation of the infusion, MABP was restored within 1–5 minutes. Rebound hypertension did not occur, although the MABP was persistently approximately 10 mmHg higher after hypotension than during the control period. However, the posthypotensive MABP was not significantly higher than the MABP before administration of dipyridamole. Heart rate, QT, and SVR returned rapidly to control levels concurrently with the restoration of MABP.

Oxygen contents, consumptions and lactate concentrations in nine patients before, during and 30 minutes after controlled hypotension are summarized in Table III.
TABLE III

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>Adenosine</th>
<th>Control 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVDO2 (mM per liter)</td>
<td>46.3 ± 2.3</td>
<td>29.3 ± 2.5*</td>
<td>46.8 ± 3.2</td>
</tr>
<tr>
<td>AVDO2 (mM per liter)</td>
<td>85.2 ± 10.5</td>
<td>58.1 ± 14.1**</td>
<td>74.9 ± 7.7</td>
</tr>
<tr>
<td>VnO2 (mM per min.)</td>
<td>220 ± 15</td>
<td>193 ± 16**</td>
<td>235 ± 16</td>
</tr>
<tr>
<td>PaCO2 (nmHg)</td>
<td>11.0 ± 9.8</td>
<td>9.75 ± 9.0</td>
<td>9.90 ± 5.2</td>
</tr>
<tr>
<td>Lactate (mmol per liter)</td>
<td>1.46 ± 0.17</td>
<td>1.73 ± 0.20</td>
<td>1.82 ± 0.20**</td>
</tr>
</tbody>
</table>

**P < 0.01
***P < 0.05, denotes significantly different from control 1.

From Table III, it can be seen that arterial oxygen tension remained unchanged during adenosine-induced hypotension. VO2 was decreased by 13±4%, with a decrease in AD7O2 of 37±5%. The arterial lactate concentration was not affected by hypotension. The cerebral AD7O2 decreased similarly by 37±13%, while the arterio-venous lactate content difference was unaltered.

After the hypotensive period, the metabolic variables returned to the control levels, except for a minor increase in the arterial lactate concentration.

The ECG the day after the operation was unchanged. The mean serum creatinine level was 83.5±4 μM before operation and 70.1±3 and 71.2±4 μM on the first 2 postoperative days.

Adenosine infusion rate was constant during the hypotension, which suggests the absence of tachyphylaxis.

Subsequent to the tests described above, a 20 mM solution of adenosine in isotonic saline was administered to 50 surgical patients employing techniques similar to those described, except that pretreatment with dipryridamole was omitted. Hemodynamic effects similar to those described above were observed at adenosine dosages of 0.2–0.35 mg per kg per minute. The enhanced cardiac output obtained with adenosine, in combination with the maintained right and left heart filling pressures, is in contrast with the hemodynamic effects of controlled hypotension with sodium nitroprusside or nitroglycerine, as is shown in Table IV.

<table>
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<th>Control 1</th>
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<tr>
<td>MABP (mmHg)</td>
<td>110 ± 16</td>
<td>95 ± 20</td>
<td>100 ± 20</td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td>20 ± 5</td>
<td>18 ± 5</td>
<td>22 ± 5</td>
</tr>
<tr>
<td>RAP (mmHg)</td>
<td>15 ± 2</td>
<td>12 ± 2</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>CI (liters per minute per meter square)</td>
<td>4.5 ± 0.5</td>
<td>3.5 ± 0.5</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>SVR (dyne-sec-cm⁻²)</td>
<td>1,200 ± 200</td>
<td>1,400 ± 200</td>
<td>1,200 ± 200</td>
</tr>
<tr>
<td>HR (beats per minute)</td>
<td>70 ± 5</td>
<td>65 ± 5</td>
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</tr>
<tr>
<td>QT (milliseconds)</td>
<td>450 ± 100</td>
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<td>QT (milliseconds)</td>
<td>450 ± 100</td>
<td>450 ± 100</td>
<td>450 ± 100</td>
</tr>
</tbody>
</table>
surgery. Accordingly, drugs with vasodilator properties, such as isoflurane and nitroprusside, have been investigated for possible use to increase myocardial blood flow and to reduce peripheral vascular resistance (after-load reduction) during such surgery; however, they have been found to have no beneficial effect with respect to coronary flow and, indeed, may reduce coronary blood flow. In contrast, adenosine administered by continuous infusion has been found very effective in increasing myocardial blood flow and, such use, is accompanied by an increase in cardiac output.

For such application, the rate of adenosine administration should be such that there is no more than a 10–20 percent reduction in blood pressure. As a general rule, this is achieved by use of rates of administration of the order of 0.05 to about 0.1 mg. adenosine per kilogram per minute. In such a case myocardial blood flow has been found to be doubled, cardiac output has been increased by 10 to 20 percent, and blood pressure has been reduced by 10 to 20 percent, without change in oxygen consumption and without ECG signs of ischemia.

EXAMPLE IV
CORONARY VASODILATION

It has been further found that when adenosine is administered by infusion at rates which do not induce significant hypotension, it has clinically useful regional effects in unanesthetized and anesthetized patients.

For example, adenosine at dosages of the order of 10 to 15 percent of hypotensive levels (e.g. 0.02 to 0.05 mg. per kg. per minute) can be a useful adjunct to coronary by-pass surgery, apparently due to a preferential coronary vasodilatation. It has been reported that coronary artery grafts occlude more frequently during the postoperative period when low graft-flow values are obtained during surgery. See Groudin et al. Circulation, 42: Suppl 3: 106–111 (1970). It has been found that low doses of adenosine administered postoperatively increase graft blood flow without significant effect on atrio-ventricular conductance. The administration of low doses of adenosine for this purpose can be carried out for as long as is necessary to afford appropriate graft flows and to reduce risk of occlusion, but ordinarily the period need not exceed 48 hours following surgery.

In a study designed to investigate the use of adenosine to inhibit occlusion of coronary grafts, nine patients (age 45–65, all taking beta-blockers) were studied during coronary artery surgery. After premedication, morphine (10–15 mg) and scopolamine (0.4–0.6 mg), anesthesia was induced by fentanyl (30 mcg/kg.b.w.), pancuronium (0.1 mg/kg b.w.) was given to facilitate endotracheal intubation. Anesthesia was maintained with fentanyl 0.5 mg/hour, N,O (50%) in oxygen and droperidol (0.1 mg/kg b.w.). During bypass thiocarbamol (5 mg/kg b.w.) was given. Nitrous oxide was not used after bypass. Extra corporeal circulation (ECC) was performed with a roller pump and a Shiley bubble oxygenator primed with crystalloid solution. ECG (modified V_{3}) and arterial line and a Swan-Ganz Catheter were used for monitoring and for hemodynamic measurements. Blood flow in bypass grafts (n=15, internal mammary and venous grafts), was measured with appropriate sized square wave electromagnetic flow probes (Nyctantron). The study was performed 20–30 minutes after the termination of ECC. After a control period (5 min), adenosine (5.3 mg/ml clinical solution) was continuously infused in a central vein in order to induce approximately 10% reduction of mean arterial blood pressure (about 30 to 50 µg per kg. per min.). Graft flow was continuously measured before and during a 10 or 30 minute infusion of adenosine and finally during the following 5 minute control period. Data are expressed as mean ±SEM and differences were tested with Student’s paired t-test against the preceding period.

The results of this study are summarized in Table V.

| TABLE V |
|-----------------|-----------------|-----------------|
| CONTROL BEFORE | ADENOSINE | CONTROL AFTER |
| Mean Arterial Pressure (mmHg) | 84 ± 3 | 74 ± 3 | 85 ± 3 |
| Heart Rate (beats/min) | 82 ± 5 | 82 ± 15 | 81 ± 6 |
| Cardiac Output (L/min) | 4.8 ± 0.4 | 5.6 ± 0.3 | 5.3 ± 0.3 |
| Pulmonary Artery Pressure (mmHg) | 16.7 ± 1.2 | 18.8 ± 1.2 | 19.9 ± 1.0 |
| Right Artery Pressure (mmHg) | 4.7 ± 0.5 | 5.3 ± 0.4 | 5.8 ± 0.7 |
| Stroke Index (mL/min/m²) | 35.6 ± 2.6 | 38.8 ± 2.0 | 39.6 ± 2.5 |
| Left Ventricular Stroke Index (mL/min/m²) | 0.44 ± 0.03 | 0.41 ± 0.03 | 0.49 ± 0.04 |
| Graft flow (mL/min) | 40 ± 5 | 77 ± 7 | 99 ± 5 |

As is evident from Table V, adenosine in a dose of 49±4 µg/kg/minute, a level which reduced mean arterial pressure 12%, increased cardiac output 12%, and doubled graft flow. At the same time, heart rate, mean pulmonary artery pressure, central venous pressure, stroke index and left ventricular stroke work index remained essentially unchanged. Graft flow rate was restored to its original value on termination of adenosine. No arrhythmias were observed.

This demonstrates that i.v. adenosine at low rates (30–50 µg per kg per min.) induces a marked and reproducible increase in graft flow without increased myocardial work, apparently due to preferential vasodilatory effect of adenosine in the coronary vasculature.

EXAMPLE V
INCREASED CARDIAC OUTPUT

As is evident from the foregoing data, intravenously infused adenosine has the ability to increase cardiac output without increasing heart work. This is in contrast to other vasodilators, such as sodium nitroprusside, which may reduce cardiac output, depending on the hemodynamic status of the patient. As a consequence, adenosine can be used to stimulate cardiac output in patients with low cardiac output states, for example, to heart surgery, infarct and the like. This apparently is due to adenosine’s ability to reduce after-load, without having significant effect on preload. In contrast, nitroprusside reduces both after-load and preload, and nitroglycerine is effective principally (90%) on reducing preload, and has only a marginal effect on after-load.

For this application, effective dosages are intermediate those used for increased graft flow and controlled hypotension. Typically the effective dose is of the order of 40–80 µg/kg/minute. The duration of treatment can be as long as required to support the heart. It also has been found that, on termination of the adenosine, cardiac output, although less than that during treatment with adenosine, frequently remains above the cardiac output prior to treatment.

In this respect, adenosine is of value as an adjunct to dopamine treatment for cardiogenic shock. Dopamine is
frequently given to patients in shock to stimulate heart action and thereby increase blood pressure. Adenosine can be administered with dopamine to modulate peripheral resistance without compromising systemic blood pressure, and thus increase cardiac output.

Adenosine is unique in its activity in this respect, because it is able to reduce after-load without significantly increasing heart rate. In contrast, agents previously used to reduce cardiac after-load, for example, hydralazine and prazosin, increase heart rate.

EXAMPLE VI

PLATELET PROTECTION DURING CARDIOPULMONARY BYPASS

Continuous infusion of adenosine also has been found of use in protecting platelets during cardiopulmonary bypass. For such use, it is desired to maintain the adenosine dosage below that affording significant vasodilation, and a rate of about 100 μg/kg/min has been found effective. In contrast, prostacyclin, a prostaglandin used to inhibit platelet aggregation, is associated with severe systemic vasodilation and hypotension during coronary bypass surgery.

Twenty-five patients scheduled for coronary artery bypass surgery were randomly assigned to two groups—one with adenosine infusion (n=13) and the other with placebo infusion (n=12).

Routine tests of coagulation status were normal in all patients, and none was taking drugs known to affect platelet function. Intravenous anesthesia was used, either high-dose fentanyl (100–150 μg/kg) or balanced anesthesia (thiopental, fentanyl, diazepam and N2O/O2).

During cardiopulmonary bypass (CPB), mean arterial blood pressure (MABP) above 70 mmHg was treated with the vasodilator sodium nitroprusside (SNP), except in the final phase or rewarming. CPB was performed with SARN roller pump and a Shiley oxygenator (100A) primed with 2000 ml crystalloid solution (75 mg heparin). The perfusion rate was kept at approximately 1.8 ml/m2 body surface. Moderate hypothermia (25°C) was induced. Cardioplegia was obtained with Ringer’s solution (with added potassium up to 20 mM). Heparin (3 mg/kg) was administered as a bolus injection before cannulation. The heparin effect was controlled by measurements of activated clotting time (ACT) (Hen’s Int. Technidyne Corp, USA). This time (ACT) was >400 seconds in all patients during CPB. At the termination of CPB, the heparin effect was antagonized with protamine (c. 1.3 mg/mg heparin). ACT was checked 10–20 min after the protamine injection. ACT values 120 sec were considered satisfactory.

Platelet count (Linsor 431A cell counter) and hematocrit were determined in arterial samples before anesthesia, after thoracotomy, during CPB at 10, 20, 40, 60, 80 and 100 min, 30 min after CPB and on the postoperative day. Platelet counts were expressed in percentage of preanesthesia levels and were corrected for hemodilution. MABP was monitored continuously via a catheter introduced in the radial artery.

Perioperative blood loss and blood transfusions could not be compared between the groups, due to the smallness of the series and the involvement of many surgeons in the study. Postoperative bleeding was measured as the blood loss from the tube drainage from the end of operation until the postoperative morning. One patient in the adenosine group was excluded because of reoperation for surgical reasons (for massive bleeding due to suture insufficiency in a graft anastomosis) within 6 hours after CPB.

Adenosine (5.3 mg/ml, clinical solution) was infused at a rate of 100 μg/kg/min into the superior vena cava throughout CPB. The adenosine dose was based on five pilot cases in which a vasodilator dose-response was observed. The highest infusion rate that did not induce systemic vasodilation was chosen for this study. In six cases the plasma adenosine levels were determined by high performance liquid chromatography (HPLC) (Predholm and Sollevi, J. Physiol. (London), 313: 351–67 (1981) in arterial and in venous (venous lines to the oxygenator) blood. The adenosine metabolites inosine, hypoxanthine and uric acid were also determined by HPLC. The samples were collected as previously described (Sollevi, Ada. Physiol. Scand., 121: 165–72 (1984) at the intervals of 10, 20, 40 and 80 minutes during CPB and 20 minutes after CPB.

Results are summarized in Tables VI and VII, below, in which data are presented as means ±SEM. Statistical significance (controls v adenosine group) was determined with Student’s t-test for unpaired data. For significance within the groups the Wilcoxon Rank Sum test was used. p<0.05 was regarded as significant.

<table>
<thead>
<tr>
<th>TABLE VI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PATIENT DATA</strong></td>
</tr>
<tr>
<td><strong>Adenosine (μg per kg per min)</strong></td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Male/Female</td>
</tr>
<tr>
<td>CPB-time (min)</td>
</tr>
<tr>
<td>Preoperative platelet counts</td>
</tr>
<tr>
<td>x 10³ cells/l</td>
</tr>
<tr>
<td>Prooperative urine production (mL/min CPB)</td>
</tr>
<tr>
<td>Postoperative blood loss (mL)</td>
</tr>
</tbody>
</table>
TABLE VII

Arterial and Venous Concentrations (μM) of Adenosine and its Metabolites (n = 6), Before, During and After Adenosine Infusion (0.1 mg/kg/min × min−1)

<table>
<thead>
<tr>
<th></th>
<th>pre-CBP</th>
<th>10'</th>
<th>20'</th>
<th>40'</th>
<th>60'</th>
<th>90' post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>0.3 ± 0.2</td>
<td>3.7 ± 1.3*</td>
<td>5.7 ± 2.1*</td>
<td>4.5 ± 1.1*</td>
<td>3.6 ± 0.8*</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Inosine</td>
<td>0.2 ± 0.1</td>
<td>0.9 ± 0.3*</td>
<td>2.4 ± 1.2*</td>
<td>1.6 ± 0.5*</td>
<td>1.6 ± 0.4*</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>3.2 ± 0.8</td>
<td>5.3 ± 1.2*</td>
<td>7.7 ± 1.4*</td>
<td>5.3 ± 1.0</td>
<td>5.0 ± 0.9</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>250 ± 30</td>
<td>260 ± 32</td>
<td>269 ± 35</td>
<td>250 ± 32</td>
<td>249 ± 30</td>
<td>260 ± 34</td>
</tr>
</tbody>
</table>

*significantly different from pre-CBP value.

As shown in Table VIII, platelet count was similar in the two groups before anesthesia and was unaltered by anesthesia and thoracotomy. In the control group the platelet count fell rapidly and markedly during the first 40 minutes of CPB and remained significantly reduced during and after CPB. During adenosine infusion the initial platelet reduction was small, and was significant only at 20 and 40 minutes on CPB. From 60 minutes to the end of CPB and at 30 minutes after CPB the platelet counts were not significantly different from those before anesthesia. Throughout CPB and 30 minutes after CPB there was significant intergroup difference in platelet counts. On the day after operation the platelet counts were not significantly different. The postoperative blood loss did not differ between the groups.

All patients were extubated within 24 hours after the operation and all recovered normally. There were no clinical signs of neurologic complications and all the patients were discharged from the hospital.

EXEMPLARY VII

ADDITION OF ADENOSINE TO CARDIOPLEGA SOLUTION

Cardioplegia is induced during open heart surgery in order to arrest the heart and to reduce myocardial oxygen consumption during cardiopulmonary bypass. This is at present generally obtained by ice-cooled solution containing high concentration of potassium (20 mmol/liter, four times the normal serum level) that is infused into the coronary vessels. It is well known that high concentrations of potassium effectively induce asystole, but also cause damage on vascular endothelium. The latter may lead to permanent stenosis of coronary vessels.

The foregoing data has clearly demonstrated in human patients that adenosine is effective as coronary vasodilator and preserves circulating platelets. Adenosine is also known to be incorporated into high energy phosphates (ATP) in various tissues. In addition, it is well known since the early work of Drury and Szent Gyorgyi (J. Physiol (London) 68:213 (1929)) that high concentration of adenosine can produce heart block.

These four effects of adenosine are all useful during the induction of cardioplegia in human patients. First, the vasodilatory effect can counteract the vasoconstrictor effect of potassium and thereby reduce the time required for administration of cardioplegia solution. This will give a more rapid cooling and thereby more rapid asystole.

Secondly, the inhibitory effect on platelet activation can prevent platelet aggregation in the coronary circulation during this cooling phase. Third, adenosine can...
Increased pulmonary resistance, e.g., pulmonary hypertension may clinically manifest as a severe disturbance of myocardial function of the right ventricle, a myocardial insufficiency, and an impaired whole body oxygenation. Increased pulmonary resistance may be chronic or acute, i.e., occur as a consequence of surgery, or pulmonary disease. The present invention is also directed to decreasing or normalizing acute pulmonary vascular resistance and pulmonary hypertension.

Pharmacological treatment of increased pulmonary vascular resistance or acute pulmonary hypertension has focused on using traditional vasodilating agents. These agents have been shown to have limited effects on pulmonary hypertension. The vasodilatory and hypotensive effects of these agents are primarily systemic, not localized in the pulmonary vasculature.

The endogenous vasodilator adenosine, in contrast to these traditional agents, has been shown to be most effective as a dilator of blood vessels in the organs in animal models as well as in humans. In animal and clinical studies, systemic vasodilator doses of adenosine have had negligible or at most, minor effect on normal pulmonary vascular resistance. Surprisingly, however, in animal models (pig) where pulmonary hypertension has been induced by hypoxic ventilation during anesthesia, intravenous adenosine administration unexpectedly produces a marked reduction in this higher-than-normal pulmonary vascular resistance. Even more surprising is the fact that this decrease in pulmonary vascular resistance occurs at a dosage of adenosine lower than that which exhibits systemic vasodilator effects. This effect is obtained at doses of adenosine that commonly do not result in detectable vasodilator effects or effects on cardiac output or arterial oxygen function.

It is believed that adenosine can exert its vasodilatory hypotensive effects in the pulmonary circulation without inducing systemic vasodilatation because of its extremely rapid elimination in the blood stream (T½ less than 10 seconds). Thus, a dose titration of adenosine can be performed, producing effects in the pulmonary vasculature without producing systemic effects at least in part because the adenosine is eliminated before it reaches the resistance vessels of the systemic vasculature.

The expected suitable infusion rate of adenosine in humans to normalize pulmonary vascular resistance is typically 10–30 micrograms per kilogram of body weight (0.010–0.030 mg per kg per minute). As a general rule, the concentration of the adenosine in the infusion solution is at least about 5 millimol (1.5 mg/ml) and may be as high as the solubility limit of adenosine (about 20 millimol or 5.5–6 mg/ml). However, in small children, the concentration of adenosine in solution may be as low as 0.1 mg/ml.

Preferably, to effectuate the maximum pulmonary hypotensive effect, the adenosine solution is administered by infusion through a catheter to maximize the exposure of the pulmonary vasculature to adenosine. This is done by infusing the adenosine solution into a central vein such as the superior vena cava, or alternatively, into the right atrium. As the adenosine solution is infused, the pulmonary vascular resistance is monitored to determine the effect of the adenosine. Infusion of adenosine is maintained until pulmonary vascular resistance returns to normal levels or until there is no further evidence of decreasing pulmonary vascular resistance. Adenosine infusion may, of course, be continued for periods of time within practical limits in particularly difficult cases of higher-than-normal pulmonary vascular resistance.

Using this selective pulmonary effect of adenosine, low infusion rates may be used for the treatment of acute postoperative pulmonary hypertension that occurs in patients after heart transplant surgery or in other surgeries.

EXAMPLE IX
DECREASING PULMONARY VASCULAR RESISTANCE IN CONJUNCTION WITH IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

Adenosine infusion as described in example VIII may also be used to normalize pulmonary vascular resistance (pulmonary hypertension) in children with idiopathic respiratory distress syndrome (IRDS).

Such treatment may be performed by infusing the low doses of adenosine as described in example VIII into the patient's pulmonary vasculature, e.g., via catheter introduced in the pulmonary artery for a period sufficient to lower pulmonary vascular resistance. The catheter's position should be distal to the ductus arteriosus. This way, adenosine does not enter the systemic circulation. Preferably, pulmonary vascular resistance should be lowered to within normal ranges. Thus, adenosine infusion should be maintained for a period sufficient to normalize pulmonary vascular resistance. This can be done by monitoring pulmonary vascular resistance during the infusion process, and maintaining infusion until pulmonary vascular resistance levels fall to normal. In difficult cases, adenosine infusion may be maintained for periods of time within practical limits.

EXAMPLE X
A METHOD OF DIAGNOSING THE OPERABILITY OF THE PULMONARY VASCULATURE IN PATIENTS EXHIBITING PULMONARY HYPERTENSION IN CONJUNCTION WITH CARDIAC SEPTUM DEFECTS

In children with myocardial septum defects (Vitium Organicum Cordis, VOC), who also exhibit pulmonary hypertension, adenosine infusion provides a means by which the value of surgically repairing septum defects can be determined. Adenosine induced reduction of pulmonary vasculature resistance may be a rapid and simplified technique for the evaluation of the usefulness of surgical repair of septum defects. This technique is a welcome diagnostic.
tool since patients with septum defects having severe morphological damage of the pulmonary vasculature caused by pulmonary hypertension would not normally be responsive to adenosine vasodilation. This condition is not improved by surgical repair of the VOC.

The methodology comprises infusing an adenosine solution (infusion rate of 0.010–0.030 mg, per kg, per minute with a concentration of about 1.5 mg/ml to about 5.5–6.0 mg/ml) into the blood stream of a patient to maximize the exposure of the patient’s pulmonary vasculature to adenosine. This can be done by infusing a solution of adenosine into a central vein, for example, the superior vena cava, or alternatively, into the right atrium. The blood pressure in the lung artery is then measured before and after administration of the adenosine as per the previously described technique in examples I and VI (cannula introduced in the artery). A decrease of arterial blood pressure is indicative of a reduction of the pulmonary vascular resistance and this, in turn, is an indication that the pulmonary vasculature will respond to surgical repair of the VOC. On the other hand, if there is no decrease in arterial pressure, then this would be indicative of morphological damage of the type that would not be improved by surgery.

An alternative to measuring a decrease in mean arterial pressure is to measure heart minute volume. In this technique, the heart minute volume is measured (same technique as described above in Example I) during administration of adenosine for a time period long enough to observe the pressure or flow change in heart volume. An increase in heart minute volume would indicate that the operability of the pulmonary vasculature had not been impaired, and that the cardiac septum defects could be corrected with surgery. No change would indicate the septum defects could not be corrected with surgery.

EXAMPLE XI

ADENOSINE IN PERCUTANEOUS TRANSLUMINAL CORONARY ANGIOPLASTY

A new method of treating coronary artery disease in human beings is effected by inserting a special catheter, equipped with an inflatable balloon, into a coronary artery which has an angiographically demonstrable stenosis. The procedure, known as percutaneous transluminal coronary angioplasty (PTCA), is executed as follows: under radiological control, the balloon of the catheter is placed in the stenosed part of the vessel. The balloon is inflated several times, each time with increasing pressures, and for a duration of 1 minute. Thereafter, the catheter is withdrawn from coronary circulation and the flow through the so treated vessel is checked by means of coronary angiography. The resultant widening of the diseased part of the coronary vessel leads to cracks in the intimal cell layer of the vessel. This trauma leads to activation of biochemical processes leading to local production of substances able to constrict the vessel, as well as activating platelets, which are circulating in the blood, in such a way that they are more easily deposited on the site of the previous stenosis. Such a platelet deposition is the first initiation of the coagulation process, which ultimately can lead to the formation of a blood clot. All these events act in conjunction to counter the intended effect of the PTCA treatment, and may ultimately lead to a re-oclusion of the vessel. During the period the PTCA procedure has been in widespread use, re-oclusions have been found to occur within 6 months of treatment in 25% or more of the cases. It is generally agreed that 5–10% or more occur in the first few days after treatment.

In order to lessen or prevent the negative effects of PTCA described above, vasodilating substances such as nitroglycerine, sodium nitroprusside, and the like, as well as platelet inhibiting substances and substances preventing blood coagulation such as acetylsalicylic acid, dipryridamol, heparin, coumarin and warfarin, have been administered to the patient before and after the procedure. However, all these substances have actions that are either too potent to be safe in conjunction with the PTCA procedure, since bleeding complications from the catheter puncture sites may occur, or too weak or unpredictable to be fully effective.

Adenosine may be used effectively in conjunction with PTCA because it possesses a unique combination of beneficial properties which all work to antagonize the complicating reactions described above. It has a potent vasodilatory effect on the coronary circulation which enables good blood flow through the treated vessel, which in turn prevents platelet deposition on the traumatized vessel site. In addition, adenosine antagonizes the action of locally produced vaso-constrictor substances. Adenosine also has an inhibiting effect on platelet aggregation, which further inhibits the chances of clot formation in the treated vessel. These effects are further enhanced by the ability of adenosine to inhibit presynaptic neural mechanisms regulating the release of catecholamines from nerve endings of the sympathetic nervous system which, as is well known, have consequences that all work for clot formation.

The adenosine dosage anticipated to be effective in this context is typically from 10 to 100 microgram of adenosine per kilogram per minute. Since the PTCA procedure is performed in an awake patient higher doses will generally not be employed, since such doses produce symptoms such as facial flushing, neck and chest oppression, palpitation and increased rate of respiration in an awake patient which, although not harmful or life-threatening, nonetheless should be avoided because they may indicate coronary occlusion.

Adenosine is preferably administered into a peripheral vein such as the femoral vein or brachial vein. Preferably, administration is begun shortly, e.g., a few minutes, before PTCA and continued for the duration of the PTCA and for several hours, e.g., 24 hours after PTCA.

The adenosine treatment may be given to patients undergoing the PTCA treatment under current medication with other anti-anginal drugs, such as adrenergic blocking drugs, calcium antagonists, diuretics, digitalis glycosides, angio-tension converting enzyme inhibitors, antihyperlipidemic drugs, nitrate compounds including nitroglycerin or other vasodilatory compounds.

EXAMPLE XII

ADENOSINE IN CORONARY THROMBOLYSIS

A recent advance in the treatment of acute myocardial infarction is by means of introducing substances in the bloodstream to dissolve the clot(s) in the coronary circulation, which in most cases are the cause of the diseased state. This procedure, which is generally referred to as coronary thrombolysis (CTL), is performed by introducing streptokinase, urokinase or tissue plasminogen activator, either intravenously or directly into the coronary circulation. All of the advantages noted above for use of adenosine in PTCA are also applicable to the CTL procedure now in use, or which may be used in the future, since the same vascular and platelet reactions which occur with PTCA also occur in CTL once the thrombolysis has been achieved. In the CTL context, however, it may be advantageous to administer the
adenosine concomitantly with the thrombolytic agent, either separately or premixed with it in a fixed solution, such premixed solution being a further aspect of this invention. When the two agents are administered separately, it is desirable but not essential that the initiation of administration of each be simultaneous. For example, administration of the adenosine may be initiated before administration of the thrombolytic agent.

In PCI, as in PTCA, adenosine may be administered intravenously at the same dosage as with PTCA. It is recognized that, due to the very brief half-life of adenosine, the dose is dependent on the site of administration. For example, a 30-milligram dose of adenosine per kilogram per minute given in the right atrium of the heart provides very similar effects to a 50-milligram dose of adenosine per kilogram per minute given in a forearm vein. It is conceivable that in CTI procedures, adenosine may be given directly in the coronary circulation. The dose then needed to achieve the same effects as described with intravenous administration would then be expected to lie in the range of 5 to 30 microgram of adenosine per kilogram per minute.

**EXAMPLE XIII**

**ADENOSINE IN THE DIAGNOSIS OF CORONARY HEART DISEASE BY RADIONUCLIDE SCINTIGRAPHY**

In the diagnosis of coronary heart disease, modern techniques include visualization of myocardial ischemia by means of injecting short-lived radio-isotopes, such as thallium-201, into the blood-stream and record, by means of a gamma-radiation detector, the activity over the heart muscle.

It has been shown that an injection of the vasodilator dipryridamol can cause about the redistribution of flow within the heart muscle so that those areas irrigated by stenosed vessels may be better visualized. The mechanism behind this is the so-called "steal" phenomenon: with a generalized maximal vasodilation of the heart muscle, relatively more blood will flow through the vessels not stenosed or constricted in any other way, thereby "stealing" the flow from the area supplied through a stenosed vessel.

Dipryridamol is an adenosine uptake inhibitor, which means it prevents adenosine from crossing the cell membranes of the red blood cells from the plasma to the interior (the normal, main pathway for adenosine elimination from plasma), thereby increasing the adenosine levels in plasma. Most data concerning the mechanism of action of dipryridamole's vasodilatory effect in fact support the view that it is solely due to adenosine vasodilation.

In a further aspect of this invention, adenosine can be used instead of dipryridamole in the diagnostic test described. It would in fact be an advantage to use adenosine instead as it can be dosed exactly and dose-titrated to a precise effect, whereas with dipryridamole, the adenosine levels are unpredictable. Thus, a safer and more reliable test can be expected if adenosine is used.

The exact dose will normally have to be titrated individually but should lie in the range of 10 to 150 micrograms per kilogram per minute.

This invention has been described in terms of specific embodiments set forth in detail herein, but it is to be understood that these are by way of illustration and the invention is not necessarily limited thereto. Modifications and variations will be apparent from the disclosure and may be resorted to without departing from the spirit of the invention as those of skill in the art will readily understand. Accordingly, such variations and modifications are considered to be within the purview and scope of the invention and the following claims.

What is claimed is:

1. A method of selectively vasodilating the arteries of a human patient, without inducing significant venous dilation and without pretreatment with dipryridamole, comprising continuously administering into the blood stream of said patient adenosine at a rate of administration of 0.35 milligrams of adenosine per kilogram body weight per minute, or less.

2. A method according to claim 1 in which adenosine is administered to an anesthetized patient undergoing surgery.

3. A method of selectively vasodilating the arteries of a human patient, without inducing significant venous dilation and without pretreatment with dipryridamole, comprising continuously administering into the blood stream of said patient by intravenous administration about 0.05 milligrams to about 0.30 milligrams of adenosine per kilogram body weight per minute.

4. A method according to claim 3 in which adenosine is administered to an anesthetized patient undergoing surgery.

5. In a surgical method carried out on a patient under general anesthesia the improvement comprising continuously administering into the blood stream of said patient adenosine in an amount sufficient to selectively vasodilate the arteries of said patient without pretreatment with dipryridamole, at a rate of administration of 0.35 milligrams of adenosine per kilogram body weight per minute, or less.

6. In a method as claimed in claim 5, the improvement comprising continuously administering into the blood stream of said patient adenosa at a rate of from 0.05 to about 0.3 milligrams of adenosine per kilogram body weight per minute.

7. A method of selectively vasodilating the arteries of a human patient, without inducing significant venous dilation and without pretreatment with dipryridamole, comprising continuously administering into the blood stream of said patient adenosine at a rate of administration of 0.01 to 0.15 milligrams of adenosine per kilogram body weight per minute.

8. A method for selectively vasodilating the arteries of an anesthetized human patient, without inducing significant venous dilation and without pretreatment with dipryridamole comprising continuously administering into the blood stream of said patient adenosine at a rate of administration of 0.35 milligrams of adenosine per kilogram body weight per minute.

9. A method for inducing a reduced afterload in the vascular system of a human without reducing the preload and without pretreatment with dipryridamole, the method comprising continuously administering into the blood stream of said patient adenosine at a rate of administration of 0.35 milligrams of adenosine per kilogram body weight per minute, or less.

* * * * *
EXHIBIT B
IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

ITEM DEVELOPMENT AB,
ASTELLAS US LLC, and
ASTELLAS PHARMA US, INC.,
Plaintiffs,

v.

SICOR, INC. and
SICOR PHARMACEUTICALS, INC.,
Defendants.

Civil Action No.: 05-0336-SLR

PLAINTIFFS’ JOINT STATEMENT REGARDING
CLAIM TERMS REQUIRING CONSTRUCTION

Pursuant to the October 12, 2005 Scheduling Order in this case, Plaintiffs Astellas US, LLC, Astellas Pharma US, Inc., and Item Development AB jointly provide this statement regarding the identification of claim terms in U.S. Patent No. 5,731,296 (“the ’296 patent”) for which the parties seek a construction by the Court. The Defendants have not provided adequate contentions in this matter on either infringement or validity issues. At this time, and in view of Defendants’ current contentions, Plaintiffs do not believe any claim construction is required for the asserted claims of the ’296 patent. However, Plaintiffs reserve the right to seek construction of terms in response to more detailed contentions or claim construction issues raised by Sicor and, in particular, any new theories of invalidity or noninfringement that Sicor may raise.
Dated: March 27, 2006

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EXHIBIT C
JOINT PROPOSED CLAIM CONSTRUCTION STATEMENT

Pursuant to the Court’s Rule 16 Scheduling Order in this matter, the parties hereby submit the following claim chart to present their respective, proposed constructions of disputed claim terms of the patent in suit, namely, U.S. Patent No. 5,731,296 ("the ‘296 patent").

Defendants, Sicor Inc. and Sicor Pharmaceuticals Inc., object to Plaintiffs’ proffer of any proposed constructions because, as will be discussed in more detail in Defendants’ claim construction brief, Plaintiffs’ proposed constructions were not timely disclosed to Defendants pursuant to Paragraph 5 of the October 12, 2005 Scheduling Order in this case.

Plaintiffs disagree that there was untimely disclosure and continue to contend that the terms for which Sicor seeks a construction should be construed according to their ordinary meaning.
**Proposed Constructions of Disputed Claim Terms in the ‘296 Patent**

<table>
<thead>
<tr>
<th>U.S. Patent No. 5,731,296</th>
<th>Item and Astellas’ Proposed Construction of Disputed Terms</th>
<th>Sicor’s Proposed Construction of Disputed Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Method of selectively vasodilating the arteries . . . without inducing significant venous dilation”</td>
<td>“Method of selectively vasodilating the arteries . . . without inducing significant venous dilation” The claim term “Method of selectively vasodilating the arteries . . . without inducing significant venous dilation” must be read as a single phrase and given its ordinary meaning. Accordingly, the phrase refers to a method of causing vasodilation characterized by selective action on arteries without inducing “significant” (i.e., an “important, weighty, or notable” amount of) vasodilation of veins. See e.g. “Selective”--of, relating to, or characterized by selection; selecting or tending to select &lt; buyers of retail stores have become more and more ~ - Glen Fowler&gt; &lt;some dyes were highly ~ in their action - S. F. Mason&gt; &lt; monetary controls may be either general or ~ - Jules Backman&gt; &lt; an exceptionally quick and ~ reader - John Mason Brown&gt;. <em>Webster’s Third New International Dictionary of the</em></td>
<td>“Selectively vasodilating the arteries” In view of the specification, this phrase is properly construed “as dilating only arteries and not veins.” See ‘296 Patent, Col. 2, lines 39-42.</td>
</tr>
<tr>
<td>“Selectively vasodilating the arteries”</td>
<td></td>
<td>“Without inducing significant venous dilation” This phrase is properly construed as “the veins are not dilated to any extent that is detectable by conventional means.”</td>
</tr>
<tr>
<td>“Without inducing significant venous dilation”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(claims 1, 3, and 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U.S. Patent No. 5,731,296</td>
<td>Item and Astellas’ Proposed Construction of Disputed Terms</td>
<td>Sicor’s Proposed Construction of Disputed Terms</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------------------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td><em>See also “Significant”--having or likely to have influence or effect: deserving to be considered: important, WEIGHTY, NOTABLE</em> &lt; even though the individual results may seem small, the total of them is ~ - F. D. Roosevelt&gt;. <em>Webster’s Third New International Dictionary of the English Language Unabridged, Volume III, S to Z</em> 2116 (Encyclopedia Britannica, Inc. 1981) (1961).</td>
<td></td>
</tr>
<tr>
<td>U.S. Patent No. 5,731,296</td>
<td>Item and Astellas’ Proposed Construction of Disputed Terms</td>
<td>Sicor’s Proposed Construction of Disputed Terms</td>
</tr>
<tr>
<td>----------------------------</td>
<td>----------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>“Inducing a reduced afterload . . . without reducing the preload”</td>
<td>“Inducing a reduced afterload . . . without reducing the preload”</td>
<td>“Inducing a reduced afterload” The phrase “inducing a reduced afterload . . . without reducing the preload” is recited in asserted claim 9 of the ‘296 patent. In view of the specification, this phrase is properly construed as equivalent to the phrase “activity of adenosine is limited to dilation of arteries.” See ‘296 Patent, Col. 2, lines 39-42.</td>
</tr>
<tr>
<td>“Inducing a reduced afterload”</td>
<td>The phrase “inducing a reduced afterload . . . without reducing the preload” must be read as a single phrase and given its ordinary meaning. Accordingly, the claim refers to a method of causing vasodilation of the arteries with little or no effect as a dilator of veins. ‘296 Patent, Col. 2, lines 39-42.</td>
<td>“Without reducing the preload” The phrase “without reducing the preload” is recited in asserted claim 9 of the ‘296 patent. In view of the specification, this phrase is properly construed as equivalent to the phrase “adenosine has little or no effect as a dilator of veins.” See ‘296 Patent, Col. 2, lines 39-42.</td>
</tr>
<tr>
<td>“Without reducing the preload” (claim 9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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Attorneys for Plaintiff
Item Development AB
EXHIBIT D
IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

ITEM DEVELOPMENT AB, ASTELLAS US LLC, and ASTELLAS PHARMA US, INC. )

Plaintiffs, )

v. )

SICOR INC. and SICOR PHARMACEUTICALS, INC., )

Defendants. )

Civil Action No. 05-336 SLR

DECLARATION OF PHILIP F. BINKLEY, M.D., M.P.H.

I, Philip F. Binkley, M.D., M.P.H., hereby submit this declaration on behalf of defendants Sicor Inc. and Sicor Pharmaceuticals, Inc. (collectively “Sicor”) concerning issues of claim construction.

I. INTRODUCTION

A. Qualifications and Credentials

1. I am currently the James Hay and Ruth Jansson Wilson Professor of Medicine in the Division of Cardiology at The Ohio State University (“OSU”), where I have been a member of the faculty since 1984. In addition to my faculty appointment, I have been the Director of Cardiovascular Research in the Division of Cardiology at OSU since 1998. I also served as the section head of heart failure and transplantation from 1998 to 2003. A complete list of my positions and professional credentials is set forth in my curriculum vitae, which is attached hereto at Tab 1.
2. I have been a practicing physician and cardiologist for over twenty-one years. In addition, I am board-certified in the fields of cardiology and internal medicine.

3. In 1976, I was awarded my Bachelors of Arts degree summa cum laude in Mathematics and Premedical Studies from Ohio Wesleyan University in Delaware, Ohio. I was awarded my Doctor of Medicine degree cum laude from The OSU College of Medicine in Columbus, Ohio in 1979. In addition, I was awarded a Master of Public Health degree, with an emphasis in biostatistics and epidemiology, from The OSU School of Public Health in 2004.

4. Following my graduation from medical school, I completed a one-year medical internship in Internal Medicine and a two year medical residency in Internal Medicine at The OSU College of Medicine. In addition, I completed a three-year fellowship in Cardiovascular Medicine in the Division of Cardiology at The OSU College of Medicine.

5. I have conducted extensive research in the field of cardiology and authored over 140 published articles in this field. One of the focuses of my research is the use of vasodilators in the treatment of congestive heart failure, and I have published investigations in which non-invasive determinations of ventricular loading conditions were performed in patients with dilated cardiomyopathy. I have also authored several chapters in medical textbooks, including a chapter in Congestive Heart Failure (Hosenpud & Greenber, eds.) that is entitled “The Non-ACE Inhibitor Vasodilators.” In addition, I have presented various abstracts at numerous conferences throughout the United States.

6. I am a member of the editorial boards of the American Heart Journal and the Journal of Cardiac Failure. In addition, I have served as a peer reviewer on a number of other journals, including Circulation, Journal of the American College of Cardiology, and Journal of Arteriosclerosis, Thrombosis, and Vascular Biology.
7. I have received numerous honors and awards in connection with my work in the field of cardiology. Representative awards include the Henry Christian Award for Clinical Research, which was presented to me by the American Federation for Clinical Research, and the Unverferth Award for Research and Career Excellence, which is given to the member of the Department of Internal Medicine who has idealized the role of clinician scholar, mentor, and investigator. Moreover, I received a Mid-Career Scientist Award in Patient-Oriented Research from the National Institutes of Health, which recognizes accomplishments in clinical research and mentorship of developing clinical scientists and provides funding for patient-oriented research, mentorship, and further career development.

8. I have served as a witness before the Cardiorenal Advisory Panel of the U.S. Food & Drug Administration, and my testimony there regarded flosequinan, a pharmacological drug that is used as a vasodilator.

9. I have consulted for a number of pharmaceutical companies, including Boots Pharmaceuticals and Otsuka Pharmaceuticals. In addition, I have received research grants from both pharmaceutical companies and governmental organizations, including Wyeth Laboratories, SmithKline Beecham, Pfizer, Myogen, the National American Heart Association, and the National Institutes of Health.

B. **Materials Considered**

10. In addition to my knowledge, training, and experience, my opinion is based on the materials cited herein, which are listed at Tab 2.

II. **BACKGROUND OF THE CASE**

11. I understand that Sicor has been sued for infringement of U.S. Patent No. 5,731,296 ("the '296 patent") based upon the filing of Sicor's Abbreviated New Drug
Application No. 77-425 ("Sicor’s ANDA") for a 3 mg/ml injectable adenosine product for use as an adjunct to thallium-201 myocardial perfusion scintigraphy in patients unable to exercise adequately. I have been informed that plaintiffs Item Development AB, Astellas US LLC, and Astellas Pharma US, Inc. (collectively “plaintiffs”) are asserting claims 1, 3, 7, and 9 of the ‘296 patent against Sicor in this action.

12. I understand that the parties dispute the construction of certain terms of the asserted claims of the ‘296 patent, and I have been asked to provide my opinion concerning the proper construction of these terms.

13. My opinions are based upon my review of the ‘296 patent, as well as my review of relevant publications and my general knowledge and experience in the field of cardiology.

14. In my opinion, a person of ordinary skill in the art to whom the ‘296 patent pertains in 1985 would be a cardiologist with a residency in internal medicine and two years of a cardiology fellowship, whose experience could also include nuclear cardiology imaging.

III. TECHNICAL BACKGROUND

15. The ‘296 patent is directed to, inter alia, the use of adenosine as a vasodilator to dilate the arteries of a human patient. In order to discuss the various claim construction issues raised in this case, I believe that a brief overview of the relevant technical subject matter would be useful.

16. Vasodilators are compounds that dilate blood vessels (e.g., veins and arteries). Different types of vasodilators may dilate either veins or arteries, or both. For example, oral nitrate compounds (e.g., isosorbide mononitrate or dinitrate) principally dilate veins, while hydralazine dilates mainly arteries and arterioles. In contrast, nitroprusside dilates both arteries
and veins. In addition, some vasodilators, including calcium channel blockers, may dilate primarily arteries but also give rise to measurable venous dilation.

17. Adenosine has been known to be an arterial vasodilator for nearly 80 years. Dipyridamole has also long been used as a selective arterial vasodilator. In fact, the administration of dipyridamole and the administration of adenosine have a final common pathway that results in the same net effect: an increase in the concentration of adenosine in the blood.

18. Changes in various hemodynamic parameters may be evaluated to determine whether arterial vasodilation has occurred following the administration of an arterial vasodilator. In particular, a physician may choose to evaluate some or all of the following parameters: systolic blood pressure (SP), diastolic blood pressure (DP), mean arterial blood pressure (MABP), cardiac output, pulmonary artery pressure (PAP), systemic vascular resistance (SVR), and pulmonary vascular resistance (PVR).

19. In contrast, measurement of a reduction in venous resistance (i.e., venodilation) is more difficult. In general, venodilation is inferred from a reduction in right atrial pressure, which would indicate decreased venous resistance and an associated decrease in venous pressure.

20. Venous capacitance and venous pressure contribute indirectly to "cardiac preload." Rough estimates of cardiac preload are usually calculated by analyzing reductions in

---

1 A 1929 publication noted that adenosine lowered general cardiac pressure, in part due to general arterial dilation. See A.N. Drury et al., The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart, J. Physiol., 68:213-237 (1929) ("Drury," attached hereto at Tab 3). By the early 1980s, the use of adenosine as a potent vasodilator was well-known and accepted in the scientific community. See, e.g., R.M. Berne, The Role of Adenosine in the Regulation of Coronary Blood Flow, Circulation Research, 47(6): 807-813 (Dec. 1980) ("Berne," attached hereto at Tab 4).

2 I understand that Dr. Sollevi uses the terms "cardiac after-load" and "cardiac pre-load" in the specification of the '296 patent. However, in my opinion, a person of ordinary skill would understand that the terms afterload and preload are usually described in terms of the left or right ventricle, and thus the use of the more general term "cardiac" is imprecise. However, for purposes of my declaration, I will use these terms in a manner that is consistent with their use in the specification.
either right atrial pressure ("RAP," with respect to right ventricular preload) or left atrial pressure ("LAP," with respect to left ventricular preload). In turn, LAP is often determined by changes in the pulmonary capillary wedge pressure ("PCWP"). A decrease in either RAP or LAP can imply a decrease in ventricular preload. A more conclusive determination of preload requires measurement of ventricular pressure/volume relationships or peak filling rates of the ventricle.

21. "Cardiac afterload" is estimated by measuring systemic arterial resistance and related hemodynamic parameters. Arterial resistance contributes to the load imposed on the ventricle during systolic contraction. Therefore, reduction of systemic vascular resistance implies a reduction in afterload. More definitive measurement of afterload requires more sophisticated analysis of ventricular pressure/volume relations. In addition, the aortic input impedance spectrum and characteristic impedance of the aorta also provide more complex (and perhaps more complete) measures of the vascular loading conditions that contribute to ventricular afterload. Both of these hemodynamic parameters require the use of high fidelity catheters and instrumentation for correct measurement.

22. To a significant degree, each of the measurements discussed in paragraph 21 contributes to an assessment of the load imposed by the arterial vasculature on the ejecting left ventricle, and therefore contributes to cardiac afterload. In addition, in certain disease states (e.g., aortic valve stenosis), the valve itself also contributes significantly to afterload.

IV. CONSTRUCTION OF DISPUTED CLAIM TERMS

23. I understand that the Court has been asked to construe the following four claim terms in this case: (1) "selectively vasodilating the arteries;" (2) "without inducing significant venous dilation;" (3) "inducing a reduced afterload;" and (4) "without reducing the preload."
A. **Claims 1, 3, and 7**

24. Claims 1, 3, and 7 of the ‘296 patent recite, *inter alia*, “a method of selectively vasodilating the arteries of a human patient without inducing significant venous dilation” by the continuous intravenous administration of adenosine. Therefore, I understand that the terms “selectively vasodilating the arteries” and “without inducing significant venous dilation” are each set forth in these claims.

25. As a preliminary matter, I understand the terms “selectively vasodilating the arteries” and “without inducing significant venous dilation” to refer to two separate properties of vasodilators. As discussed above, some arterial vasodilators (e.g., calcium channel blockers) dilate primarily the arteries, but also dilate veins to an appreciable extent. In contrast, other vasodilators (e.g., hydralazine) dilate only arteries, with no detectable venous dilation. Therefore, in my opinion, a person of ordinary skill in the art in 1985 would not consider the terms “selectively vasodilating the arteries” and “without inducing significant venous dilation” to refer to a single concept, and instead would view these terms as representing two independent effects of vasodilators.

1. **“Selectively Vasodilating The Arteries”**

26. A person of ordinary skill in the art in 1985 who had reviewed the ‘296 patent would understand the phrase “selectively vasodilating the arteries,” as recited in claims 1, 3, and 7, to refer to an effect that may result from the continuous intravenous administration of a vasodilator.

27. Moreover, a person of ordinary skill would understand that the specification of the ‘296 patent had defined the phrase “selectively vasodilating the arteries” to mean that adenosine dilates only arteries, and does not dilate veins. The specification states as follows:

   [A]denosine has been found to have the following characteristics . . .
It has selective vasodilation activity . . . That is, its activity is limited to dilation of arteries . . .

See col. 2, lines 37-41. In other words, and in my opinion, a person of ordinary skill would understand the specification to expressly limit the physiological effects of adenosine to arterial dilation, and that adenosine therefore does not also dilate veins.

2. **“Without Inducing Significant Venous Dilation”**

28. A person of ordinary skill in the art who had reviewed the ‘296 patent would understand the phrase “without inducing significant venous dilation,” as recited in claims 1, 3, and 7, to refer to another effect that may result from the continuous intravenous administration of a vasodilator.

29. A person of ordinary skill of the art would understand the meaning of the phrase “without inducing significant venous dilation” in view of the context in which that phrase was used in the specification of the ‘296 patent. The specification states as follows:

> [A]denosine has been found to have the following characteristics . . . .

That is, its activity is limited to the dilation of arteries and it has little or no effect . . . as a dilator of veins.

See col. 2, lines 37-42. The phrase “has little or no effect on veins” would thus be understood in a manner consistent with the statements immediately preceding that phrase in the specification:

> “[Adenosine’s] activity is **limited to dilation of arteries** . . .” (see id.) (emphasis added).

30. I note further that a person of ordinary skill in the art in 1985 would understand the term “significant” to have various meanings within the field of cardiology. For example, “significant” could refer to clinical significance, which is any observation in the condition of a patient or population of patients that would be regarded as having practical meaning in terms of symptoms or disease. The term “significant” could also refer to physiological significance, which is a change in the function of some organ system in a patient or experimental model (e.g.,
a decrease in arterial pressure would reflect a “physiologically significant” change in the arterial vasculature). In addition, the term “significant” could also refer to statistical significance, which represents the probability that a change in a given value is greater than would be expected by chance.

31. In the context of the patent specification, however, a person of ordinary skill in the art in 1985 would understand the term “significant” to mean “detectable,” given that the term “significant” is used to compare a measurable effect on arterial dilation to the lack of an effect on venous dilation. A person of ordinary skill in the art would subsequently conclude that adenosine would not cause any dilation of veins to an extent that was detectable or inferred by conventional means (e.g., measurement of a change in right atrial pressure following adenosine administration).

B. Claim 9

32. Claim 9 of the ‘296 patent recites, inter alia, “a method for inducing a reduced afterload in the vascular system of a human patient without reducing the preload” by the continuous intravenous administration of adenosine. Therefore, I understand that the terms “inducing a reduced afterload” and “without reducing the preload” are each set forth in this claim.

33. As a preliminary matter, the terms “inducing a reduced afterload” and “without reducing the preload” refer to two separate physiological concepts. While a person of ordinary skill in the art would understand that “afterload” and “preload” are interrelated, such a person would know that afterload and preload reflect different aspects of the “load” encountered by the ventricles at different times in the cardiac cycle. Therefore, a person of ordinary skill in the art in 1985 would not consider the terms “inducing a reduced afterload” and “without reducing the
preload” to refer to a single event, but instead would view them as referring to two independent occurrences.

1. "Inducing A Reduced Afterload"

34. As stated above, determination of afterload is a complex process that may be estimated by measuring systemic arterial resistance and related hemodynamic parameters, or calculated somewhat more accurately by measuring the aortic input impedance spectrum and characteristic impedance of the aorta. Thus, a general definition for the term afterload may be defined in different ways and with varying degrees of complexity and accuracy by the scientific community, a fact that would be known by a person of ordinary skill in the art in 1985.

35. However, the specification defines the term “afterload” in the context of the ‘296 patent. In particular, the specification states as follows:

[A]denosine has been found to have the following characteristics . . . .

It has selective vasodilation activity, in that its effect is limited to cardiac after-load effect. That is, its activity is limited to dilation of arteries . . . .

See col. 2, lines 37-41.

36. In my opinion, the specification oversimplifies the complex nature of the term “afterload,” and its unsupported discussion of this term is not sufficient to draw certain conclusions – including that the effect of adenosine is “limited to cardiac after-load effect” (col. 2, lines 39-40). However, in this case, I understand these terms within the context of the specification, and as Dr. Sollevi has defined them.

37. Furthermore, person of ordinary skill would understand that the use of the limiting phrase “in that its” means that “afterload effect” should be equated with “selective vasodilation activity.” And as discussed above, the specification defines “selective vasodilation activity” to mean that adenosine dilates only arteries, not veins.
38. Therefore, and in my opinion, the phrase “inducing a reduced afterload” would be understood by a person of ordinary skill the art in the context of the ‘296 patent to mean “limited to dilation of arteries.”

2. “Without Reducing The Preload”

39. As stated above, estimation of preload is a complicated process. In fact, “preload” has been and is still regarded as a concept that does not have a universal definition:

The term preload is intimately linked to the Frank-Starling concept, although no exact definition of preload is universally accepted. Most authorities define preload as the actual sarcomere stretch that exists at the end of diastole. However, others define preload as the force that causes this sarcomere stretch. The difference in definition can lead to real discrepancies in the concept of preload.


40. However, the specification defines “preload” in the context of the ‘296 patent. In particular, the specification states as follows:

[A]denosine has been found to have the following characteristics . . . .

That is, its activity is limited to dilation of arteries and it has little or no effect on cardiac preload, i.e., as a dilator of veins.

See col. 2, lines 37-42. A person of ordinary skill in the art would be forced to look to the specification for the meaning of the term “preload,” given that the term “preload” is regarded by persons of ordinary skill as a concept that does not have a universal definition.

41. In my opinion, the specification oversimplifies the complex nature of the term “preload,” and its unsupported discussion of this term is not sufficient to draw certain conclusions – including that adenosine “has little or no effect on cardiac preload” (col. 2, lines
41-42). However, in this case, I understand this term within the context of the specification and as Dr. Sollevi has defined it.

42. Therefore, and in my opinion, the phrase "without reducing the preload" would be understood by a person of ordinary skill in the art in the context of the '296 patent to mean that adenosine does not dilate veins in a manner that can be detected or inferred by conventional means.

V. CONCLUSION

43. I hereby declare that the foregoing is true and correct to the best of my knowledge.

[Signature]

Date 8/23/06

Philip F. Binkley, M.D., M.P.H.
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Ohio State University:  M.D.  Cum Laude 1979
The Ohio State University School of Public Health:  Master of Public Health with Emphasis in Biostatistics and Epidemiology  December, 2004

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Fellowship, Ohio State University, Division of Cardiology, Columbus, Ohio 1982-1985
Clinical Instructor, Ohio State University Department of Medicine, Columbus, Ohio 1982-1985
Assistant Professor of Medicine, Ohio State University, Division of Cardiology, Columbus, Ohio 1985-1991
Associate Professor of Medicine, Ohio State University, Division of Cardiology, Columbus, Ohio 1991-1997
Professor of Medicine, Ohio State University, Division of Cardiology, Columbus, Ohio 1997 - Present
Director, Cardiovascular Research, Division of Cardiology, Ohio State University 1998 - Present
Section Head, Heart Failure/Transplantation Division of Cardiology, Ohio State University 1998 – 2003
Graduate Faculty—Biomedical Engineering and Department 1999-Present
Pathology—Advisor Status

The James Hay and Ruth Jansson Wilson Professor of Medicine, Division of Cardiology, 2000-Present
Ohio State University
Vice Chair for Academic Affairs, Ohio State University 2002-Present

Associate Director of Training and Faculty Development,
the Davis Heart and Lung Research Institute 2002- Present

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COMMITTEES - Department/Division

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2. Division of Cardiology Fellowship Committee - 1985 to present
3. Division of Cardiology Research Committee - 1990 to present
4. Department of Medicine Research Advisory Committee - 1990 to present
5. Chairman Department of Medicine Research Advisory Committee - 1993 to present
6. Department of Medicine Promotion and Tenure Committee – elected 1998
7. Chairman Department of Medicine Research Committee - 1998 to present
8. Chairman Department of Medicine Promotion and Tenure Committee -elected 7/1999

College
Roessler Committee on Student Research
Medical Scientist Training Program Committee
College of Medicine Research Committee
College of Medicine General Research Committee - 1999 to present
College of Medicine Appointments, Promotion & Tenure Committee – 2005 to present

Professional


8. Executive Committee - Board of Trustees. Franklin County, American Heart Association. 1991 to present.

9. Chairman - Central Ohio Service Area Research Committee. The Ohio Affiliate of the American Heart Association. 7/92 to present.


11. Board of Trustees - The Ohio Affiliate of the American Heart Association. 6/92 to present.


16. Chairman - Volunteer Resources Committee - Franklin County Division of the American Heart Association. 1995 to present.


20. Secretary - Board of Directors, Ohio-West Virginia Affiliate of the American Heart Association. 1997 to present.

22. American Heart Association the Ohio Valley Affiliate - President Elect 7/1999 to present

23. Member North American Steering Committee of the NIH sponsored STICH Trial 2003 to present.
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Central Society for Clinical Research - Elected 1993
American Heart Association Council on Circulation
Heart Failure Society of America - Elected 1996

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Journal of the American College of Cardiology
American Heart Journal
Diabetes
Journal of Arteriosclerosis, Thrombosis, and Vascular Biology
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Pi Mu Epsilon Mathematics Honorary - Ohio Wesleyan University
Lease Prize in Mathematics
Phi Beta Kappa - Ohio Wesleyan University (Junior elect)
Cum Laude - Ohio State University College of Medicine
AOA - Ohio State University
Central Ohio Heart Chapter Fellowship - 1983-1984
Central Ohio Heart Chapter Fellowship - 1984-1985
Philip F. Binkley

(Franklin County Fellow)
Young Investigator of the Central Ohio Heart Chapter of American Heart Assoc. 1986-1987
Young Investigator of the Central Ohio Heart Chapter of American Heart Assoc. 1987-1988
Clinical Associate Physician of the General Clinical Research Center of the National Institutes of Health - 1987-1990
Nominated as 1993 Physician of the Year by the Ohio Affiliate of the American Heart Association
Listed in Best Physicians in Midwest, Specialist in Heart Failure, 1996
Henry Christian National Award For Clinical Research - Presented by the American Federation for Clinical Research at its Annual National Scientific Meetings 1996, Washington, DC
Mid-Career Investigator Award in Patients Oriented Research (K-24) Granted by the NIH (see grants and awards)
Selected as “The Best Doctors in America” in 2002
Unverferth Award for Research and Career Excellence, 2002
Elected to Phi Kappa Phi Graduate Honorary Society 2003
Faculty mentor to Soma Mandal—winner of the 2004 Denman Research Day Award for Health Sciences Research (the Denman awards are the premiere undergraduate research awards of the Ohio State University).

GRANTS AND AWARDS

1. Central Ohio Heart Chapter. "Aortic Impedance in CHF: Response to Therapeutic Agents", 7/1/85 - 12/31/86. $18,000.00

2. Pfizer Pharmaceuticals. Analysis of Impedance in a Congestive Heart Failure Population. Pfizer Scholar Award application. 6/86 (not funded).

3. University Seed Grant: Aortic Impedance in Congestive Heart Failure: Response to Therapeutic Agents: 7/1/85-6/30/87. $7,100.00

4. Central Ohio Heart Chapter: Impedance in CHF: Response to Therapeutic Agents", 7/1/86-6/30/87. $18,270.00 Grant.

5. Young Investigator Award/Central Ohio Heart Chapter. Impedance in Congestive Heart Failure: Response to Therapeutic Agents: 7/1/86-6/30/87. $26,000.00 Stipend.

6. Central Ohio Heart Chapter, Influence of Isordil and Hydralazine on Circulatory Power, 1/1/88-6/30/88. $23,000.00 Grant.

7. Young Investigator Award/Central Ohio Heart Chapter. Influence of Isordil and Hydralazine on Circulatory Power. 7/1/88-6/30/87. $25,000.00 Stipend.

8. Central Ohio Heart Chapter, Total Artificial Heart: Central & Peripheral Vascular Effects.
Kevin Murray - PI, Philip Binkley - CoI. 7/1/87-6/30/88. $22,475.00

9. Central Ohio Heart Chapter. Doppler Indices of Diastolic Function: Effects of Heart Rate. Steven Hirsch - PI, Philip Binkley - Sponsor, 7/1/87-6/30/88. $18,000.00 Stipend.

10. National Institutes of Health. Specialized Center of Research in Heart Failure. Determinants of Vasodilator Efficacy in Heart Failure. 5/88 (approved but not funded)

11. Central Ohio Heart Chapter. Total Artificial Heart: Central & Peripheral Vascular Effects. Kevin Murray - PI, Philip Binkley - CoI. 7/1/88-6/30/89. $22,475.00

12. Central Ohio Heart Chapter. Diastolic Function in Cardiac Transplant Recipients and its Relation to Allograft Rejection, Assem Farhat - PI, Philip Binkley - Sponsor, $15,000.00 7/1/88-12/31/89

13. Central Ohio Heart Chapter, Total Hydraulic Load in Congestive Heart Failure - (Supplemental to National American Heart Association Grant) - 7/1/88-6/30/89. $15,000.00


15. Clinical Associate Professor Award, through General Clinical Research Center of the National Institutes of Health. 7/1/87-6/30/89. $96,400.00 Grant. 3rd Year Competitive Application. $40,000.00 Stipend.

16. Wyeth Laboratories. An Open Label Study of the Effects of Anaritide on Central Hemodynamics and the Regional Blood Flow in Patients with Severe Decompenated Congestive Heart Failure. 1/1/87-12/30/90. $20,000.00

17. National Institutes of Health, General Clinical Research Center - Clinical Associate Physician Award. 3rd Year Competitive Application. Approved and renewed 7/1/89-6/30/90. $48,200.00

18. American Heart Association - National Chapter, Total Hydraulic Load in Congestive Heart Failure - 7/1/88-6/30/91. $60,000.00


20. Ohio Affiliate of the American Heart Association: Autonomic Control of Ventricular-Vascular Coupling in Congestive Heart Failure. 7/1/90-6/30/91. $19,972.00

21. Ohio Affiliate of the American Heart Association: Fellowship Award to Greg Eaton, M.D., Philip Binkley,MD. Faculty Sponsor. 7/1/90-6/30/91. $20,000.00

22. Ohio Affiliate of the American Heart Association: Autonomic Control of Ventricular-Vascular Coupling in Congestive Heart Failure. (2nd year approval) 7/91-6/92. $30,000.00
23. Smith Kline & French: A Double-Blind Positive Controlled Crossover Assessment of Renal Response to Intravenous Infusion of Corlopam Compared to Sodium Nitroprusside at Doses That Increase Cardiac Outpatient in Patients with Congestive Heart Failure. 7/1/90-6/30/91. $169,244.00

24. Boots Pharmaceuticals. Spectral Analysis of Heart Rate Variability in a Population of Patients Treated with Vasodilator Flosequinan Compared to a Placebo Treated Population. 10/90-5/91. $45,000.00

25. Ohio Affiliate of the American Heart Association: Selective Autonomic Modulation of Ventricular Coupling in Congestive Heart Failure. 7/92-6/93. $30,000.00

26. Smith-Kline Beecham - "A Double-Blind Positive Controlled Cross-Over Assessment of Renal Responses to Intravenous Infusion of Corlopam Compared to Sodium Nitroprusside in Doses that Increase Cardiac Output in Patients with Congestive Heart Failure" 1990-1991 Total Award: $169,244

27. Merck Sharp and Dohme - "Open label Pilot Study of the Hemodynamic Effects of Intravenous Enalaprilat Administered as an Extended Infusion to patients with Heart Failure: 1991 Total Award:$70,500

28. Merck Sharp and Dohme - Multicenter Double-Blind Randomized Parallel Multiple-Dose Placebo Controlled Study of Hemodynamic and Clinical Effects of Mk-954 (Losartan, DUP753) in Patients with Congestive Heart Failure" 1992 Total Award: $35,571

29. Otsuka Pharmaceuticals - "A Phase 2 Stepwise Open Titration Study of the Pharmacodynamics and Pharmacokinetics of OPC-18790 in Subjects with Heart Failure: 1992-1993 Total Award:$120,500


31. Wyeth-Ayerst - "A Randomized Multi-Center Study Comparing the Efficacy and Safety of Intravenous Milrinone and Intravenous Nitroglycerin in Patients with Severe Heart Failure" 1993 Total Award: $134,432

32. Smith-Kline and Beecham - "Prospective Randomized Evaluation of Carvedilol on Symptoms and Exercise in Heart Failure: 1993-1994 Total Award: $87,000

33. Otsuka Pharmaceuticals - "A Randomized Multiple Dose Study of the Chronic Administration of Vesnarinone (OPCC-8212) in Heart Failure" 1993-1994 Total Award: $31,500

34. Boots Pharmaceuticals - Autonomic Response to Flosequinan in Congestive Heart Failure - A Placebo Controlled Analysis. Philip F. Binkley – PI. 6/92-7/93. $14,000.00

35. Boots Pharmaceuticals - Autonomic Response to Sibutramine in Normal Subjects. Philip F. Binkley - PI. 7/93-12/93. $11,500.00

37. Ohio Affiliate of American Heart Association. Nagaraja HN-Principal Investigator. Philip Binkley Co-Investigator. Markov Chain Modeling as Novel Means to Assess Heart Rate Variability. 7/1/93-6/30/95. $30,000.00 per year.

38. Pfizer Pharmaceuticals--The influence of Amlodipine on Autonomic Tone in Patients with Congestive Heart Failure--$43,000

39. Merck Sharp and Dohme--Influence of Losartan on Autonomic Tone in Congestive Heart Failure--Dr. Binkley Director of Core Laboratory For Spectral Analysis of Data Acquired in International Trial--$17,000.

40. Merck Medical School Grant. Influence of an Angiotensin II Antagonist on Large Vessel Compliance and Autonomic Tone in the Early Stages of Ventricular Failure. $30,000.00

41. Ohio Affiliate of the American Heart Association Fellowship Restoration of Baroreflex Sensitivity with Augmented Myocardial Stimulation in Congestive Heart Failure. Steven Boyer, M.D., Fellow Awardee, **Philip F. Binkley, M.D., Mentor** 1994 - 1995 $34,020.00


43. Ohio Affiliate of the American Heart Association Grant in Aid. Differential Regional Conduit Vessel Response to Ventricular Dysfunction in a Paced Canine Model of Heart Failure. Gregory Eaton PI, **Philip F. Binkley CoI.** 1994 - 1996. $90,000.00

44. National American Heart Association. Mechanisms Governing Ventrículoarticular Coupling in Congestive Heart Failure. **Philip F. Binkley PI.** 7/93-6/96. $120,000.00


46. Parke-Davis- "A 12-Week, Double-Blind, Placebo-Controlled, Multicenter Study of Oral YM087 (CI-1025) to Assess Functional Capacity in Patients With Class III Chronic Heart Failure (Protocol 1025-014)" 1999-2000 **Philip Binkley OSU PI.** Total Award: $ 67,368

47. Parke-Davis- "A Double-Blind, Placebo-Controlled Study of the IV Dose Response of YM087 on Cardiopulmonary Hemodynamics in Patients with Class III/IV Heart Failure." 1999-2000 **Philip Binkley OSU PI** Total award $97,700

48. Immunex- "Multicenter Double-Blind, Randomized, Placebo-Controlled, Phase II/III Study of the Effects of Recombinant Human Tumor Necrosis Factor Receptor (p75): Fc Fusion Protein (Etanercept) on Improvement in Patients with Chronic Heart Failure" 1999-2000 **Philip Binkley OSU PI** Total award $87,000
49. Astra- "Candesartan Cilexetil (Candesartan) in Heart Failure Assessment of Reduction in Mortality and Morbidity (CHARM)" 1999- 2001 Philip Binkley OSU PI Total Award $100,000


51. National Institutes of Health Mid Career Investigator Award in Patient Oriented Research (R-21). National Institute of Health. Depsipeptide: A Novel Histone Deacytlase Inhibitor in Leukemia. John Byrd, Philip Binkley (CoI). 1/1/02-12/31/03. $656,098.00 total award.


53. National Institute of Health. Pharmacogenetic Antiplatelet Strategies in CHD Patients (K23) Glenn Cooke PI, Philip Binkley-Mentor. 12/01/00-11/30/05. $670,339.00 total award.


56. State of Ohio. BRTT Tobacco Grant, Cardiovascular Bioengineering Enterprise. Mauro Ferrari PI, Philip Binkley, CoI. 02/01/2003 - 01/31/2006. $6,500,000.00 total award.


58. CHF Solutions—The Impact of Ultrafiltration verses Standard IV Diuretic Therapy on Heart Rate Variability in Congestive Heart Failure. 06/09/2005 - 06/30/2006 Philip F. Binkley, PI. $47,195.


60. Myogen, Inc. Study of oral enoximone versus placebo in advanced chronis heart failure subjects. Philip F. Binkley, PI. 11/15/01 - 06/30/06. $129,781 total reimbursements to date.

61. Myogen, Inc. Enoximone plus extended-release metoprolol succinate in subjects with advanced chronic heart failure (EMPOWER) Philip F. Binkley, PI. 10/01/03 - 06/30/06. $11,667.50 total reimbursements to date.

62. Myogen, Inc. An open label enoximone extension study for subjects who clinically deteriorate
during the enoximone withdrawal phase (Phase B) of EMPOWER (MY-023) Philip F. Binkley, PI. 10/18/04 - 09/15/06. $5,005.00 total reimbursements to date.

63. National Institutes of Health. Iron and atherosclerosis (R21), Subha Raman PI, Philip Binkley, CoI. 08/01/2005 - 07/31/2006. $411,125.00

64. National Institutes of Health. Eliminating Barriers to Effective Training in Clinical Investigation (T32). Philip F. Binkley, PI. 09/30/05 - 08/31/10. $3,348,925 total award.

65. National Institutes of Health. Ohio valley heart failure clinical research network (U01) William Abraham PI, Philip Binkley, CoI. 07/01/06 - 06/30/11. $2,518,254, pending


PUBLICATIONS


33. Binkley PF, Murray KD: Circulatory Assist Devices: Advances and Limitations in Their Short and Long Term Use for the Therapy of Ventricular Failure. Current Opinion in


46. Binkley PF, Boudoulas H: The Measurement of Myocardial Inotropy in Cardiotonic Drugs:


50. **Binkley PF**: Featured Research and Update on Congestive Heart Failure Mortality Trials Presented at the 1990 Scientific Session of the American Heart Association. Congestive Heart Failure Index and Reviews III(Supplement):48; December 1990.


58. Cody RJ, Haas GJ, **Binkley PF**, Caper Q, Kelley R: Plasma Endothelin Correlates with the Extent of Pulmonary Hypertension in Patients with Chronic Congestive Heart Failure. Circulation 1992;85:504-509.


72. Eaton GM, Cody RJ, Binkley PF: Increased Aortic Impedance Precedes Peripheral


82. Panina G, Khot UN, Nunziata E, Cody RJ, **Binkley PF**: Assessment of Autonomic Tone Over a Twenty-Four Hour Period in Patients with Congestive Heart Failure: Relation Between Mean Heart Rate and Measures of Heart Rate Variability. The American Heart Journal. 1995 April; 129 (4):748-840.


85. Eaton GM, Cody RJ, Nunziata E, **Binkley PF**: Early Left Ventricular Dysfunction Elicits


97. Binkley PF: Heart Rate Variability: Two Eras of Investigation (Editorial). The Journal of

98. Van Fossen DB, Binkley PF: Validation of Doppler Flow Velocities in Patients With Left Ventricular Dysfunction (in submission).


118. Philip F. Binkley, Ywen Liu-Stratton, Patty S. Hatton, Glen Cooke. A Polymorphism of the Endothelial Nitric Oxide Synthase Gene is Associated with Increased Sympathetic Drive in Patients With Congestive Heart Failure. JACC 2001;87(2)supplA:1197-44.

119. Cooke GE, Liu-Stratton Y, Ferketich AK, Frie DJ, Magorien RD, Bray PF, Moeschberger ML, Binkley PF, Goldschmidt-Clermont PJ. Effect of P1^ on Platelet Inhibition by Aspirin, Clopidogrel, or their Combination. May 2001 (JACC in revision)


122. Khouri S, Ryan J, Ferketich A, Binkley PF. Detection of Ischemia in patients with Ischemic


ABSTRACTS AND PRESENTATIONS


5. Sullivan M, Binkley PF, Unverferth D, Leier CV: Pharmacologic Intervention of


at the 1988 Scientific Sessions of the American College of Cardiology, Atlanta, GA.


35. **Binkley PF**, Van Fossen DB, Leier CV, Cody RJ: Age as a Determinant of Large Vessel


Annual Scientific Session of the American College of Cardiology, Atlanta, Ga.


meeting of the Midwest Section of the AFCR, Chicago, Illinois.


86. Binkley PF, VanFossen DB, Haas GJ, Cody RJ: Persistence of Abnormal Ventriculoarterial Coupling and Decreased Conduit Vessel Compliance Despite Peripheral Vasodilatorion with Hydralazine and Nitroglycerin in Congestive Heart Failure. Journal of the American College of Cardiology, February 1994 382A. Presented at the 43rd Scientific Sessions of the American College of Cardiology, Atlanta, GA.


91. Binkley PF, Panina G, Nunziata E, Hatton PS, Khot UN, Reed DE, Cody RJ: Angiotensin Converting Enzyme Inhibition does not Restore Normal Circadian Variation of parasympathetic Tone in Congestive Heart Failure. Clinical Research


94. Eaton GM, Boyer ST, Cody RJ, **Binkley PF**: Conduit Vessel Compliance as a Determinant of Contractile Performance in Paced Canine Model of Congestive Heart Failure. Journal of Investigative Medicine 1995;43(Sup 3):452A


98. **Binkley PF**, Panina G, Nunziata E: Low Frequency Heart Rate Variability as a Marker of Disease Progression in Congestive Heart Failure. Presented at the 68th Scientific Sessions of the American Heart Association, Anaheim, California.


120. Presented at the American Heart Association 71st Scientific Sessions, Dallas, TX, November 8-11, 1998.

121. Binkley PF, Eaton GM, Nunziata E, Leier CV: Decreased Baroreflex Stimulation by


138. **Binkley PF**, Nunziata E, Hatton P, Leier CV. The Positive Inotropic Agent...
Levosimendan Mediates Increased Cardiac Output without Progression of Sympathovagal Imbalance in Patients with Heart Failure. Circulation 2000 102(18):SII;720.


152. Ferketich AK, Binkley PF. "Depression, Gender and C-Reactive Protein: Results from NHANES III. American Journal of Epidemiology, 2003; 157:S215.


155. Ferketich AK, Binkley PF. Depression, Gender and C-Reactive Protein: Results from NHANES III. American Journal of Epidemiology, 2003; 157: S215


161. Ferkeitch AK, Binkley PF. Psychological Distress and Cardiovascular Disease: Results from the 2002 National Health Interview Survey (NHIS). Presented at the 2004 National Meeting of the Heart Failure Society of American, Toronto, Canada.

162. Ferkeitch AK, Binkley PF. Heart Failure and Inflammation: Results from the Third National Health and Nutrition Examination Survey (NHANES III). Presented at the 2004 National Meeting of the Heart Failure Society of American, Toronto, Canada.


164. Farra Y, Binkley PF. The Relation Between Plasma BNP level and the Length of Hospital Stay in Patients with Congestive Heart Failure. Presented at the 2004 National Meeting of the Heart Failure Society of American, Toronto, Canada.


**CONFERENCES**

**Selected Invited Presentations**


6. Visiting Professor - McGill College of Medicine, the Royal Victoria Hospital, Montreal, Canada. March, 1991. 1) Research Experience in the Measurement of Pulsatile Hydraulic Load. 2) Medical Faculty Conference - The Evolution of Therapy for Congestive Heart Failure.


8. Merck, Sharpe and Dohme National Heart Failure Investigator's Conference - The Use of Spectral Analysis to Desired Changes in Autonomic Tone Associated with ACE Inhibition, May 1991.


10. Visiting Professor - University of Chicago Division of Cardiology, September 1991 1) Current Theory of the Pathophysiology and Management of Congestive Heart Failure. 2) Ventriculoarterial Coupling - Its Role in the Evolution of Circulatory Failure.


15. Mortality Trials in Congestive Heart Failure and Cardiorenal Effects of ACE Inhibitors. Riverside Methodist Hospital, Columbus, Ohio: April 1992.


18. Current Management of Congestive Heart Failure: A Case Oriented Approach. Medicine Today - The Case Western Reserve University, Department of Medicine, November 13, 1992.

20. Spectral Analysis of Heart Rate Variability as a Tool for Investigation of Autonomic Tone in Congestive Heart Failure. Presented at the Ohio State University Seminar Series in Physiology, The Ohio State University, Department of Physiology, May 1993.


26. Strategies for the Management of Chronic Ventricular Failure. Park Hospital, Columbus, OH. August 1993.

27. Assessment of Autonomic Tone of the Cardiovascular System - Neurology Grand Rounds. The Ohio State University Hospitals, Columbus, OH. August 1993.


30. Medical Grand Rounds, Ohio State University Medical Center: Heart Rate Variability, Noise or Music? October 6, 1994.


33. Use of Echocardiography and hemodynamic Monitoring in the Management of Acute Heart Failure. OSU Division of Cardiology, Update on Heart Failure. June 1995.

35. New Concepts in the Management of Hypertension. Grand Rounds Union County Mercy Hospital, Marysville, Ohio. August 9, 1995 (10 attendees)

36. Congestive Heart Failure. The Columbus Board of Health "To Your Health" Public Education Television Network. July, 1994


40. Use of Heart Rate Variability to Assess Autonomic Tone in Clinical Investigation. Clinical Sciences Lecture; Medtronic, Inc. Minneapolis, Minnesota, July 9, 1996. (40 attendees)

41. Early Autonomic Changes in the Evolution of Congestive Heart Failure. International Conference on Clinical Cardiology, Athens, Greece, April 18, 1996.(300 attendees)


43. Mechanisms and Therapy for the Management of Congestive Heart Failure. National Meeting of the American College of Sports Medicine, Cincinnati, Ohio, May 26, 1996. (300 attendees)


45. Depression, Gender and C-Reactive Protein: Results from NHANES III. 130th Annual Meeting of the American Public Health Association, November 19, 2003.


47. A Case of Medical Equipoise. Stich Investigators’ Meeting; the 2004 National Meeting of the Heart Failure Society of America, Toronto, Canada.

2. Division/Department


2. Efficacy of Isosorbide Dinitrate in Congestive Heart Failure. Expert Witness for State of Ohio


3. Community Instruction


4. Update on Management of Congestive Heart Failure, Columbus Community Hospital, July 18, 1995.


4. Invited Chair of Scientific Sessions


GRADUATE STUDENTS, MEDICAL STUDENTS, AND FELLOW TRAINEES


22. Saleem Ahmed - Boroncle Scholar in Research in Internal Medicine, 1995-1996.


24. Talal Attar, MD - Resident, Internal Medicine, Cardiovascular Research, 1996.

25. Sammer Khouri - MD-Fellow in Cardiology, Research Advisee, 1999 to present.

26. Talal Attar, MD - Fellow in Cardiology, Research Advisee, 1999 to present.


28. Jeanette Pohorence - PhD Candidate in Pathology, Advisor, 2000 to present.
TAB 2
Materials Considered

U.S. Patent No. 5,731,296;


A.N. Drury et al., *The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart*, J. Physiol., 68:213-237 (1929);