Platelet Function Studies in Heart Disease

VI. Enhanced Platelet Aggregate Formation Activity in Congestive Heart Failure: Inhibition by Sodium Nitroprusside

JAWAHAR MEHTA, M.D., AND PAULETTE MEHTA, M.D.

SUMMARY We studied 11 patients with congestive heart failure and 10 normal volunteers for in vivo platelet aggregate formation activity. The patients with heart failure had significantly \((p < 0.01)\) more circulating platelet aggregates than the normal volunteers. During sodium nitroprusside infusion, the number of circulating platelet aggregates declined to normal levels and in vitro platelet aggregation responses to epinephrine and adenosine diphosphate were also suppressed significantly \((p < 0.01)\). This was associated with a 30% decline in systemic vascular resistance and a 28% increase in cardiac output. In other in vitro experiments, sodium nitroprusside was found to have direct, dose-related platelet aggregation inhibitory actions. This study suggests that an increase in vascular resistance in certain heart failure patients may in part be related to an increase in circulating platelet aggregates. Direct inhibition of platelet aggregation by sodium nitroprusside may be a mechanism of its beneficial effects in heart failure.

PATIENTS WITH VASCULAR insufficiency have been shown to have increased in vivo platelet aggregate formation activity\(^1,2\) and increased in vitro platelet aggregation in response to epinephrine and ADP.\(^3,4\) Several investigators have suggested that the occlusion of small vessels by platelet aggregates may be responsible for tissue ischemia, injury and death.\(^5-9\) Most patients with congestive heart failure have increased vascular resistance as either a primary or a secondary event.\(^10,11\) Whether platelet aggregate formation activity is in any way related to the increase in vascular resistance in these patients is not known. The use of vascular resistance-lowering agents like nitroprusside has been beneficial in the treatment of patients with heart failure.\(^11,12\) Sodium nitroprusside is thought to be effective by its direct dilatory actions on vascular smooth muscle,\(^14\) resulting in decreased vascular resistance and improved cardiac function. Recently, the platelet aggregate inhibitory effects of nitroprusside have also been recognized.\(^15-17\)

This study was designed to evaluate in vivo platelet aggregate formation activity in patients with congestive heart failure. We also studied the effects of intravenous administration of nitroprusside on platelet aggregate formation.

Materials and Methods

Patient Population

Eleven patients (age 24–59 years, mean 48 years) who had chronic congestive heart failure, documented by clinical, roentgenologic and hemodynamic criteria, and were undergoing nitroprusside therapy, form the basis of this report. Heart failure in eight patients was due to ischemic cardiomyopathy and in three to long-standing valvular disease. Ten had been previously treated with digitalis and diuretics, and one had received only diuretic therapy. All patients were in a stable state and had no recognizable acute illness. Blood studies were also performed on 10 normal, healthy volunteers (age 22–41 years, mean 32 years) as control. None of the patients or the healthy volunteers had taken aspirin or other agents known to alter platelet function in the preceding 2 weeks.

Hemodynamic Studies in Heart Failure Patients

After an informed consent detailing the procedure was obtained, a triple-lumen, flow-directed catheter was positioned in the pulmonary artery to measure pulmonary artery and wedge pressures and cardiac output. An arterial cannula was also inserted in the brachial artery to record the systemic pressure. The details of the hemodynamic measurements have been previously reported.\(^12\) After control hemodynamics were recorded, a nitroprusside infusion was started at 10 \(\mu g/min\). The dose was gradually increased in 10-\(\mu g/min\) increments every 10 minutes until a significant change in hemodynamics (a reduction in pulmonary wedge pressure to 15 mm Hg or a 50% increase in cardiac output or a decline in the systolic blood pressure to 100 mm Hg) occurred. The nitroprusside infusion was then gradually discontinued.

Platelet Studies

Blood was collected from all patients in the control state and during nitroprusside infusion. Blood was col-
lected once from all but three of the normal volunteers, from whom blood was collected twice at a 1-hour interval for validation of platelet studies.

Blood (9 ml) was collected from a previously unpunctured peripheral vein using a large-lumen needle (#18), each time by the same person and at a steady rate. The blood was immediately transferred into polypropylene tubes containing 1.0 ml of 3.8% sodium citrate. From these samples, platelet-rich plasma (PRP) was obtained by centrifugation at 150 g for 8 minutes at room temperature. The remaining blood was spun at 5,000 g for 30 minutes at room temperature. The platelet counts in PRP were adjusted at 300,000/mm³ by adding PPP as required. The platelet aggregation studies were performed in duplicate within 2 hours of blood collection, as described earlier.18 Samples of 0.45 ml PRP were prewarmed for 1 minute by stirring in siliconized cuvettes placed in a Biodata Aggregation Profiler (Biodata Corporation, Philadelphia, Pennsylvania) before addition of aggregation stimulating agents. These agents included epinephrine 55.0 μM, adenosine diphosphate (ADP) 1.0 μM and ADP 2.0 μM. ADP was kept frozen as a stock solution of 20 μM and was diluted at the time of testing. Maximal aggregation (PA max) was read as the percentage increase of light transmission observed at 5 minutes from the graphic record after the aggregation agent was added. The rate of aggregation (PAp/min, %) and the extent of aggregation (PAp max, %) of the primary wave were also calculated as shown in figure 1.19 PPP was set as equal to 100% light transmission.

The method used to determine microthrombus formation activity was similar to that described by Wu and Hoak.1 1 2 20 Samples of exactly 0.5 ml each were withdrawn into plastic syringes containing 2.0 ml of buffered EDTA or buffered EDTA and formalin. The samples were taken in duplicate, gently tilted and allowed to stand for 15 minutes at room temperature. The four tubes were then centrifuged simultaneously at 150 g for 8 minutes to obtain PRP. The platelet counts on both PRP samples were determined under phase microscopy, and the results were expressed as follows:

Microthrombus Index (MTI) = Platelet count in EDTA/formalin PRP

Platelet count in EDTA PRP

This method is based on the idea that platelet aggregates, when present, will be fixed in the EDTA/formalin mixture and centrifuged down. Therefore, the platelet count of the PRP solution will be reduced. However, in the syringe with EDTA alone, platelet aggregates are broken. Therefore, the ratio approaches one when there are no aggregates, but drops below one if aggregates are present. The buffered EDTA and formalin solution was prepared by adding 3.0 ml of 0.077 M EDTA, 5.0 ml of 4% formalin, and 2.0 ml of concentrated (10X) phosphate-buffered saline solution (PBS) to 10.0 ml of distilled water. The buffered EDTA solution was prepared by adding 3.0 ml of 0.077 M EDTA and 5.0 ml of concentrated (10X) PBS to 12.0 ml of distilled water. Both solutions were isotonic, with a pH of 7.4. All the solutions were freshly prepared before blood collection.

In Vitro Studies

Sodium nitroprusside, obtained as anhydrous powder (Roche Labs, Nutley, New Jersey), was diluted with 5% dextrose in water (DSW) to achieve different concentrations and was used within 4 hours while being protected from exposure to light. The PRP obtained from 10 normal volunteers was incubated at 37°C for 3 minutes and either 0.1 ml of DSW or 0.1 ml of DSW and nitroprusside was added. The platelet aggregation studies were performed as described above, using epinephrine, ADP 1.0, 2.0 and 4.0 μM and collagen 0.13 mg as aggregating agents.

Calculations

The average of the duplicate values from each patient and the control subjects was used for cal-

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**Figure 1.** A typical platelet aggregation curve after the addition of aggregating agent. The method for measuring the rate of aggregation for the primary wave (PAp/min), the maximal extent of aggregation for the primary wave (PAp max), and the maximal extent of total platelet aggregation (PA max) are shown.
culations. The mean and standard error of the mean were determined for PAp max, PAp/min, PA max and MTI. The t test for paired and unpaired data was used to determine the level of significance of the difference between the mean values. A p value < 0.05 was considered statistically significant.

Results

Microthrombus Index in Heart Failure Patients and Normal Volunteers

The values for each person studied are shown in figure 2. MTI varied in heart failure patients from 0.22-0.98 (mean 0.65 ± 0.06), which was significantly (p < 0.001) lower than that in normal volunteers (range 0.86-1.02, mean 0.94 ± 0.02). The duplicate values in all subjects agreed closely (mean variation 0.04). MTIs repeated 1 hour apart in three healthy volunteers were similar (mean variation 0.03).

Sodium Nitroprusside Infusion

Hemodynamic Effects

The hemodynamic effects of nitroprusside are shown in table 1. The heart rate and arterial pressure were unchanged, while cardiac output increased 28% and pulmonary artery and capillary wedge pressure declined 29% and 36%, respectively (all p < 0.001). These hemodynamic changes were observed in each patient studied. The dose of nitroprusside required varied from 40–90 µg/min (mean 55 µg/min).

Platelet Aggregation

Nitroprusside infusion inhibited both the primary and secondary waves of aggregation: PAp/min, PAp max and PA max were significantly lower compared with pre-nitroprusside infusion (table 2, fig. 3). Platelet aggregation was inhibited in all subjects studied.

Microthrombus Index

During nitroprusside therapy, MTI in heart failure patients increased significantly (p < 0.001) to 0.88 ± 0.66 (range 0.41–1.20) (fig. 4). This increase in MTI was observed in each patient. MTI during nitroprusside therapy in heart failure patients was similar (p = NS) to that in normal volunteers.

Direct Effect of Nitroprusside on In Vitro Platelet Aggregation

In vitro addition of nitroprusside depressed the platelet aggregation response to the aggregating agents (table 3, fig. 5). The inhibition of platelet aggregation was dose-related. The maximal platelet aggregation was decreased at low concentrations, as were PAp/min and PAp max. Higher concentrations of nitroprusside further reduced aggregation responses. Lower concentrations of nitroprusside (0.1 µg/ml) inhibited ADP-induced aggregation, but a higher concentration (1.0 µg/ml) was required to significantly inhibit epinephrine-induced platelet aggregation. The effect of larger amounts of the aggregating agent (ADP) could be blocked by larger amounts of nitroprusside.

Discussion

Several investigators have shown that in vivo platelet aggregate formation activity is increased in ischemic disorders such as coronary heart disease, peripheral vascular disease and cerebrovascular in-
Increased platelet aggregate formation also occurs in diabetes mellitus, when associated with vascular injury. Our study shows that certain patients with congestive heart failure and increased vascular resistance also have a significant increase in circulating platelet aggregates compared with healthy volunteers. Nitroprusside therapy in heart failure patients leads to suppression of in vivo platelet aggregate formation and in vitro aggregation responses. Simultaneously, cardiovascular hemodynamics improve and resistance in systemic and pulmonary vascular beds declines.

The technique for quantitating circulating platelet aggregates used in this study is simple. The results are reproducible if the blood is withdrawn at a steady rate and the test is completed within an hour of blood collection. The circulating platelet aggregates are not affected by age, smoking, aspirin and anticoagulant drugs.

Increased platelet aggregation has been implicated in the genesis and propagation of arterial and venous thrombi. A similar increase in platelet aggregation has been observed in certain hypertensive persons with increased systemic vascular resistance. The observation of platelet aggregates in the microvasculature prompted several investigators to propose a role of platelets in limiting blood flow. An increase in in vivo platelet aggregate formation activity as observed in our patients could similarly impede blood flow in heart failure. A decrease in flow and a subsequent increase in vascular resistance caused by platelet aggregates may lead to further deterioration of left ventricular function and a low cardiac output state in these patients. On the other hand, an increase in circulating catecholamines due to a poor circulatory state in congestive heart failure per se may be responsible for enhanced platelet aggregate formation. Further, as platelets come in contact with collagen in the vascular bed, platelet aggregates may be formed in response to release of thromboxane A2. Whether increased aggregate formation is a primary or secondary phenomenon is not clear from our studies. Nevertheless, the formation of a large number of platelet aggregates in the intravascular space may lead to decreased blood flow and increased vascular resistance. These aggregates could later disaggregate and maintain platelet count. Similar mechanisms have been proposed by Moschos et al.

### Table 2. Effect of Nitroprusside Therapy on in Vitro Platelet Aggregation Response in Heart Failure Patients

<table>
<thead>
<tr>
<th>Aggregation stimulating agents</th>
<th>Epinephrine (µM)</th>
<th>ADP (0.1 µM)</th>
<th>ADP (2.0 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAp/min (C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>35 ± 7</td>
<td>59 ± 8</td>
<td>89 ± 11</td>
</tr>
<tr>
<td>NP</td>
<td>25 ± 6*</td>
<td>40 ± 7*</td>
<td>66 ± 6*</td>
</tr>
<tr>
<td>PAp max (C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>24 ± 4</td>
<td>34 ± 6</td>
<td>61 ± 7</td>
</tr>
<tr>
<td>NP</td>
<td>24 ± 5</td>
<td>24 ± 6*</td>
<td>46 ± 8*</td>
</tr>
<tr>
<td>PA max (C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>71 ± 9</td>
<td>47 ± 10</td>
<td>75 ± 7</td>
</tr>
<tr>
<td>NP</td>
<td>49 ± 11*</td>
<td>29 ± 8*</td>
<td>60 ± 10*</td>
</tr>
</tbody>
</table>

*p <0.01 NP vs C.

Abbreviations: ADP = adenosine diphosphate; PAp/min = rate of aggregation for the primary curve; PAp max = maximal platelet aggregation; C = control; NP = during nitroprusside infusion.
to explain the platelet kinetics during passage through an obstructed coronary vascular bed.

Sodium nitroprusside is often used to treat hypertension and congestive heart failure. The beneficial effects of nitroprusside are supposed to be related to smooth muscle relaxation and the subsequent decline in vascular resistance. The platelet aggregation inhibition and a decrease in in vivo aggregate formation activity by nitroprusside observed in our patients may also have salutary effects on vascular resistance. A decrease in in vivo platelet aggregate formation activity could result in fewer aggregates in the microcirculation and decreased impedance to left ventricular outflow. In vitro experiments by us and others show that nitroprusside has direct and potent inhibitory effects on platelet aggregation that seem to occur at a concentration of nitroprusside used clinically in most heart failure patients. The mechanism of platelet aggregation inhibition proposed by Saxon and Kattlove is direct inhibition of thrombosthenin, a platelet smooth-muscle-like protein. An improved circulatory state during nitroprusside therapy in heart failure patients per se may also be related to a decline in platelet aggregate formation because of decreased stasis of blood.

Whether the effects of nitroprusside on platelet aggregate formation are coincidental or an important

**Figure 4.** Effect of sodium nitroprusside infusion on the platelet microthrombus index in heart failure patients. The index increased in each.

**Table 3.** Direct Effect of Various Concentrations of Nitroprusside on Platelet Aggregation Response

<table>
<thead>
<tr>
<th>Aggregation stimulating agents</th>
<th>Epinephrine 55.0 μM</th>
<th>ADP 1.0 μM</th>
<th>ADP 2.0 μM</th>
<th>ADP 4.0 μM</th>
<th>Collagen 0.13 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PAP/min (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D5W</td>
<td>39 ± 5</td>
<td>69 ± 5</td>
<td>72 ± 6</td>
<td>100 = 8</td>
<td>41 ± 8</td>
</tr>
<tr>
<td>NP 0.01 μg/ml</td>
<td>42 ± 6</td>
<td>44 ± 7*</td>
<td>70 ± 9</td>
<td>96 ± 4</td>
<td>27 ± 5*</td>
</tr>
<tr>
<td>0.1 μg/ml</td>
<td>40 ± 4</td>
<td>40 ± 6*</td>
<td>54 ± 8*</td>
<td>97 ± 9*</td>
<td>20 ± 4*</td>
</tr>
<tr>
<td>1.0 μg/ml</td>
<td>19 ± 2*</td>
<td>9 ± 2*</td>
<td>16 ± 7*</td>
<td>34 ± 8*</td>
<td>4 ± 2*</td>
</tr>
<tr>
<td>10.0 μg/ml</td>
<td>10 ± 2*</td>
<td>2 ± 1*</td>
<td>3 ± 1*</td>
<td>22 ± 7*</td>
<td>2 ± 1*</td>
</tr>
<tr>
<td>100.0 μg/ml</td>
<td>4 ± 1*</td>
<td>0 ± 1*</td>
<td>0 ± 1*</td>
<td>11 ± 2*</td>
<td>1 ± 1*</td>
</tr>
<tr>
<td><strong>PAP max (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D5W</td>
<td>28 ± 3</td>
<td>31 ± 4</td>
<td>38 ± 3</td>
<td>82 ± 5</td>
<td>74 ± 6</td>
</tr>
<tr>
<td>NP 0.01 μg/ml</td>
<td>29 ± 4</td>
<td>20 ± 4*</td>
<td>38 ± 5</td>
<td>73 ± 5</td>
<td>63 ± 6*</td>
</tr>
<tr>
<td>0.1 μg/ml</td>
<td>17 ± 2*</td>
<td>10 ± 4*</td>
<td>8 ± 5*</td>
<td>26 ± 7*</td>
<td>11 ± 5*</td>
</tr>
<tr>
<td>1.0 μg/ml</td>
<td>13 ± 2*</td>
<td>3 ± 1*</td>
<td>4 ± 2*</td>
<td>20 ± 2*</td>
<td>5 ± 2*</td>
</tr>
<tr>
<td>10.0 μg/ml</td>
<td>11 ± 2*</td>
<td>0 ± 1*</td>
<td>0 ± 1*</td>
<td>10 ± 2*</td>
<td>2 ± 1*</td>
</tr>
<tr>
<td><strong>PA max (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D5W</td>
<td>91 ± 3</td>
<td>65 ± 11</td>
<td>65 ± 7</td>
<td>91 ± 3</td>
<td>81 ± 5</td>
</tr>
<tr>
<td>NP 0.01 μg/ml</td>
<td>93 ± 2</td>
<td>45 ± 10*</td>
<td>69 ± 8</td>
<td>80 ± 7</td>
<td>71 ± 8†</td>
</tr>
<tr>
<td>0.1 μg/ml</td>
<td>85 ± 4</td>
<td>44 ± 6*</td>
<td>47 ± 9*</td>
<td>52 ± 4*</td>
<td>57 ± 8*</td>
</tr>
<tr>
<td>1.0 μg/ml</td>
<td>34 ± 7*</td>
<td>16 ± 8*</td>
<td>26 ± 8*</td>
<td>31 ± 8*</td>
<td>15 ± 6*</td>
</tr>
<tr>
<td>10.0 μg/ml</td>
<td>24 ± 6*</td>
<td>6 ± 4*</td>
<td>22 ± 6*</td>
<td>20 ± 4*</td>
<td>12 ± 7*</td>
</tr>
<tr>
<td>100.0 μg/ml</td>
<td>24 ± 1*</td>
<td>6 ± 1*</td>
<td>12 ± 5*</td>
<td>20 ± 3*</td>
<td>6 ± 3*</td>
</tr>
</tbody>
</table>

* p < 0.01 (NP vs D5W).
† p < 0.05 (NP vs D5W).

Abbreviations: ADP = adenosine diphosphate; PAP/min = rate of aggregation for the primary curve; PAP max = extent of aggregation for the primary curve; PA max = maximal platelet aggregation; D5W = 5% dextrose in water; NP = nitroprusside.
mechanism of its beneficial actions in heart failure is not clear. In a recent report, Jackson et al. have demonstrated beneficial actions of prostaglandin A1, a platelet aggregation inhibitor, in patients with heart failure. In our preliminary studies, we have found that nitroglycerin, which has minimal arteriodilatory actions, has only slight platelet aggregation inhibitory effects, which may in part account for the absence of increase in cardiac output in heart failure patients given nitroglycerin. Several other vasodilators are being used in the management of heart failure patients. Some of these agents may have platelet aggregation inhibitory actions as well. To define the precise relationship between platelet aggregation, vascular resistance and heart failure, further studies are warranted.

In summary, our observations suggest increased in vivo platelet aggregate formation activity in certain heart failure patients. Sodium nitroprusside therapy results in decreased platelet aggregate formation activity toward levels seen in normal persons. These platelet aggregation inhibitory actions of nitroprusside could complement its smooth muscle relaxation effect, resulting in decreased vascular resistance and increased cardiac output in patients with heart failure.

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Figure 5. Effect of in vitro addition of 5% dextrose in water (D5W) and gradually increasing amounts of nitroprusside (NP) on platelet aggregation responses. NP produced a dose-related inhibition of platelet aggregation. ADP = adenosine diphosphate.
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