Reduced Albuminuria with Sarpogrelate Is Accompanied by a Decrease in Monocyte Chemoattractant Protein-1 Levels in Type 2 Diabetes

Susumu Ogawa, Takefumi Mori, Kazuhiro Nako, Tsuneo Ishizuka, and Sadayoshi Ito

Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University School of Medicine, Sendai, Japan

Background and objectives: Sarpogrelate has been shown to reduce albuminuria in diabetic nephropathy. For examination of whether this is based on the same mechanisms as angiotensin II receptor blockers or thiazolidinedione, effects of sarpogrelate on atherosclerotic inflammatory molecules and their relations to albuminuria in patients who had diabetes and had already been treated with angiotensin II receptor blockers and with or without thiazolidinedione were examined.

Design, setting, participants, & measurements: Forty patients who had diabetes with nephropathy and arteriosclerosis obliterans and had already been treated with angiotensin II receptor blocker (n = 40) or sarpogrelate (300 mg/d; n = 20) or aspirin group (100 mg/d; n = 20). Plasma monocyte chemoattractant protein-1 and urinary albumin-to-creatinine ratio and monocyte chemoattractant protein-1 were measured at baseline and 16 wk after administration.

Results: Only the sarpogrelate group showed increases in plasma adiponectin and decreases in both plasma and urinary monocyte chemoattractant protein-1 and albumin-to-creatinine ratio levels. Moreover, percentage change of monocyte chemoattractant protein-1 level correlated positively to that of albumin-to-creatinine ratio. Even when the sarpogrelate group was further divided into two groups with (n = 9) or without thiazolidinedione (n = 11), changes in monocyte chemoattractant protein-1 or albumin-to-creatinine ratio did not differ.

Conclusions: Sarpogrelate can reduce albuminuria and plasma and urinary monocyte chemoattractant protein-1 levels while increasing plasma adiponectin in diabetic nephropathy. These effects seem to be mediated via mechanisms that are different from those of angiotensin II receptor blocker or thiazolidinedione.


Cardiovascular injury is the most important factor that determines the life prognosis of the individual with diabetes. There is an urgent need to elucidate its pathogenesis and establish effective treatment modalities. In recent years, serotonin (5-hydroxytryptamine [5-HT2A]) receptor has been shown to play an important role in cardiovascular injury (1). We previously showed that sarpogrelate, a 5-HT2A receptor antagonist, inhibits production of thromboxane A2 (TXA2) and suppresses urinary albumin excretion (albumin-to-creatinine ratio [ACR]) in patients with diabetic nephropathy (2). Because nephropathy correlates strongly with cardiovascular disease (3), it is speculated that sarpogrelate may have both direct and indirect cardiovascular protective effect.

Stimulation of 5-HT2A receptors by serotonin induces expression of TGF-β, a key mediator of fibrosis, in mesangial cell via the signaling pathway of the following order: G-protein–coupled 5-HT2A receptor, protein kinase C, NADPH oxidase, reactive oxygen species (ROS), mitogen- and extracellular signal-regulated kinase (MEK), extracellular signal–regulated kinase (ERK), and TGF-β (4). 5-HT has been shown to enhance production of type IV collagen by human mesangial cells, and its production is mediated by activation of protein kinase C and subsequent increase in active TGF-β (5). Recently, it was reported that the enzymatic pathway mediating serotonin synthesis is present in renal cortex, proximal tubules, glomerular epithelial cells, and mesangial cells (6). In the kidney, serotonin activates ERK and increases the expression of connective tissue growth factor and TGF-β, which are key mediators of extracellular matrix accumulation, and stimulates the expression of vascular endothelial growth factor (VEGF) in podocytes (6); therefore, sarpogrelate may have a potential of suppressing the progression of diabetic glomerulosclerosis.

Inflammation plays an important role in the pathophysiology of cardiovascular injuries. A variety of inflammation markers have been established, including monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-6, and high-sensitivity C-reactive protein (hsCRP). Especially, MCP-1 plays an important role in the recruitment of monocytic cells to the site of inflammation. Local accumulation of macrophages plays an important role in diabetic nephropathy (7), and MCP-1 plays a key role in this accumulation of macrophages (8,9). Moreover, urinary MCP-1 strongly reflects the pathology of nephropathy (10–14). There is a possibility that serotonin activates janus kinase/signal transducer and activators of the transcription.
Adiponectin has cardioenovascular protective actions, and its reduction is closely related to cardioenovascular injuries. Both thiazolidinedione (TZD) and angiotensin II (AngII) type I receptor blockers (ARB) have been reported to elevate plasma adiponectin levels and to lower MCP-1 levels (16–20). Administration of sarpogrelate was also reported to elevate plasma adiponectin levels (21,22); however, it is unclear whether this effect is mediated by a common mechanism or by an entirely different mechanism from that of ARB or TZD. This is an intriguing issue, considering that all three drugs (sarpogrelate, ARB, and pioglitazone) reduce urinary albumin excretion; however, no previous studies have investigated this issue clinically. In this study, we administered sarpogrelate to patients who had albuminuria and type 2 diabetes and were already receiving treatment with ARB and with or without pioglitazone and examined plasma and urinary inflammation markers and their relations to ACR. We also examined whether sarpogrelate’s effects were different between patients who were treated with pioglitazone and those without.

**Concise Methods**

The patients had type 2 diabetes and met the following requirements: They had (1) at least one of the following symptoms: Pain in the lower limbs, cold sensation, intermittent claudication, impalpable pedal artery, or ankle brachial index <0.9; (2) maximum internal carotid medial thickness >1.0 mm; (3) ACR >30 mg/g creatinine; (4) glycosylated hemoglobin <8.0%; (5) BP <180/110 mmHg; and (6) no serious retinopathy. Those who had their drugs changed or had been hospitalized during the past year for any reason were excluded from the study. Those who had already been treated with antiocoagulants (including aspirin) were also excluded. Sarpogrelate is indicated for the treatment of arteriosclerosis obliterans (ASO) in Japan. Because sarpogrelate is an antiarteriosclerosis drug that has a powerful action of suppressing platelet aggregation, 100 mg/d aspirin, which has also an antiplatelet action, was used as the control drug (23). Aspirin is used widely in patients with arteriosclerosis and is also a leading drug for suppressing platelet agglutination; therefore, our study targeted patients who had diabetic nephropathy and were experiencing ASO as a complication and used aspirin as the control drug to examine whether antiplatelet actions are involved in the mechanisms of sarpogrelate-induced reduction of ACR and inflammations.

This study was carried out as a single-blind clinical study. The patients were randomly divided into the sarpogrelate (300 mg/d) administration group and the aspirin (100 mg/d) administration group. Routine examinations were performed before administration and 16 wk later. In addition, plasma and urinary MCP-1, plasma IL-6, serum hsCRP, and plasma adiponectin were measured. Plasma and urinary MCP-1 and plasma IL-6 were measured by MCP-1 ELISA Kit (R&D Systems) and IL-6 ELISA Kit (R&D Systems, Minneapolis, MN), respectively, and adiponectin was measured by ELISA using the Adiponectin ELISA Kit (Ohtsuka Pharmaceutical, Tokyo, Japan).

This study was conducted after obtaining the informed consent from all patients, and the study protocol was approved by the ethics committees of Tohoku University Hospital. Because the trial was started before the clinical study registry has been widely used and took us more time than was initially anticipated, no registration was carried out in this study.

**Statistical Analyses**

The study sample size of 40 patients provided 80% power with 0.05 to detect a ~25% difference between each group in the percentage change in urinary ACR from baseline to 16 wk (assuming an SD for percentage changes in ACR of 30%).

Normally distributed data were represented as means ± SEM. Because levels of adiponectin, MCP-1, IL-6, and ACR did not show normal distributions, the actual measurement values were given as a median (range), and their logarithmic converted values (that were distributed normally) were given as means ± SEM. We also calculated the change from baseline to the end of observation in individual cases (the value after treatment − the value before treatment), as well as the percentage change rate ([the value after treatment − the value before treatment]/the value before treatment × 100). These values were also normally distributed and were expressed as means ± SEM. The majority of normally distributed data were tested, using either the paired or unpaired t test. Actual measurement values of MCP-1, IL-6, ACR, and adiponectin were compared using the Mann-Whitney U test (intragroup comparison) or the Wilcoxon signed rank test (comparison between before and after treatment). Percentage changes were compared using the χ² test. Correlations were determined by the Spearman rank correlation test. P < 0.05 were regarded as significant.

**Results**

Table 1 shows demographic data at baseline. No differences were seen between the sarpogrelate and aspirin groups (Table 1). The oral antihypertensive drugs that were administered in the sarpogrelate group and the aspirin group are as follows (the number of patients in the sarpogrelate group versus the aspirin group): ARB (20 versus 20), angiotensin-converting enzyme inhibitor (ACEI; 12 versus 13), calcium channel blockers (15 versus 17), and diuretics (5 versus 4). Thus, all of the patients had already been treated with ARB. The number of ACEI-treated patients did not differ between the sarpogrelate and aspirin groups. The oral hypoglycemic agents that were administered were as follows: Sulfonylurea drugs (9 versus 10), biguanide (8 versus 8), α-glucosidase inhibitors (13 versus 15), TZD (pioglitazone; 9 versus 10), and insulin (14 versus 13). The antihyperlipidemic drugs that were administered were as follows: Statins (7 versus 8) and fibrate (4 versus 3). No differences between the two groups were seen in ankle brachial index, pulse wave velocity, or internal carotid medial thickness. No differences were seen between the two groups in the degree of retinopathy or smoking (Table 1). No significant differences were seen between the sarpogrelate and aspirin groups in body mass index, glycosylated hemoglobin, systolic BP, diastolic BP, serum total cholesterol, triglyceride, or HDL cholesterol at baseline and 16 wk later. There were no differences in the baseline values between the two groups, and the two groups also showed no changes in these covariates (Table 2). The estimated GFR was calculated, using the Modification of Diet in Renal Disease (MDRD) calculation formula: estimated GFR = 0.741 × 175 × (age − 0.203) × (Creatinine − 1.154) × (0.742, if female) (24). The values of hsCRP in the sarpogrelate group were 0.15 ± 0.04 and 0.16 ± 0.04 mg/dl at baseline and 16 wk, respectively, whereas corresponding values in the aspirin group were 0.15 ± 0.03 and
Table 1. Baseline characteristics and before and after values for covariates of study patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sarpogrelate</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>11/9</td>
</tr>
<tr>
<td>Age (yr; mean ± SD)</td>
<td>68.7 ± 1.51</td>
</tr>
<tr>
<td>Duration of diabetes (yr; mean ± SD)</td>
<td>11.2 ± 1.51</td>
</tr>
<tr>
<td>DR</td>
<td>16</td>
</tr>
<tr>
<td>Smoker (current/former)</td>
<td>2/6</td>
</tr>
<tr>
<td>OHA</td>
<td>18</td>
</tr>
<tr>
<td>Insulin</td>
<td>14</td>
</tr>
<tr>
<td>ABI (mean ± SD)</td>
<td>0.91 ± 0.02</td>
</tr>
<tr>
<td>PWV (cm/s; mean ± SD)</td>
<td>1972 ± 48.35</td>
</tr>
<tr>
<td>MaxIMT (mm; mean ± SD)</td>
<td>1.74 ± 0.20</td>
</tr>
</tbody>
</table>

*ABI, ankle brachial index; DR, diabetic retinopathy; OHA, oral hypoglycemic agents; max IMT, maximum intima media thickness; PWV, pulse wave velocity.

Table 2. Before and after values for covariates of study patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sarpogrelate</th>
<th>Aspirin</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>24.10 ± 0.56</td>
<td>24.00 ± 0.63</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.70 ± 0.11</td>
<td>6.60 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>135.00 ± 5.52</td>
<td>134.00 ± 5.75</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>71.50 ± 2.76</td>
<td>71.20 ± 2.85</td>
<td>NS</td>
</tr>
<tr>
<td>Cr (mg/dl)</td>
<td>0.76 ± 0.05</td>
<td>0.77 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>72.40 ± 6.13</td>
<td>71.90 ± 6.67</td>
<td>NS</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>194.00 ± 8.77</td>
<td>192.00 ± 9.04</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>113.00 ± 11.22</td>
<td>124.00 ± 15.62</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>47.60 ± 2.37</td>
<td>46.40 ± 2.49</td>
<td>NS</td>
</tr>
<tr>
<td>ANP (pg/ml)</td>
<td>36.10 ± 5.61</td>
<td>38.60 ± 6.32</td>
<td>NS</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>55.90 ± 4.83</td>
<td>58.20 ± 5.21</td>
<td>NS</td>
</tr>
<tr>
<td>hsCRP (mg/dl)</td>
<td>0.154 ± 0.04</td>
<td>0.158 ± 0.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Data are means ± SEM. ANP, atrial natriuretic peptide; BMI, body mass index; BNP, brain natriuretic peptide; Cr, serum creatinine; DBP, diastolic BP; eGFR, estimated GFR; HbA1c, glycosylated hemoglobin; hsCRP, high-sensitivity C-reactive protein; SBP, systolic BP; TC, serum total cholesterol; TG, serum triglyceride.

0.14 ± 0.03 mg/dl (mean ± SEM), respectively, showing no significant changes in either group.

Table 3 shows the changes in adiponectin, MCP-1, IL-6, and ACR in the sarpogrelate and aspirin groups. In the sarpogrelate group, the adiponectin level rose significantly from 7.9 to 12.0 μg/ml (median). The absolute and percentage changes were 4.39 ± 1.10 μg/ml and 79.6 ± 29.1%, respectively. These values were significantly greater than those in the aspirin group (0.59 ± 0.45 μg/ml and −7.3 ± 5.38%). MCP-1 level decreased significantly in the sarpogrelate group from 153 to 114 pg/ml (median), and the absolute and percentage changes were −35.0 ± 8.55 pg/ml and −20.1 ± 6.21%, respectively. These decreases were significantly greater than those in the aspirin group (−21.4 ± 13.4 pg/ml and −9.09 ± 8.33%).

No differences were seen between the two groups in the ACR values at baseline. The ACR in the sarpogrelate group decreased significantly from 267 to 204 mg/g creatinine (median). The absolute and percentage changes were −129 ± 43.2 mg/g creatinine and −22.8 ± 9.35%, respectively. These decreases were significantly greater than those in the aspirin group (34.7 ± 25.5 mg/g creatinine and 15.5 ± 11.6%); however, IL-6 levels showed no significant changes in either the sarpogrelate or the aspirin group.

Table 4 shows the changes in adiponectin, MCP-1, and ACR in the sarpogrelate group, according to the presence and absence of pioglitazone treatment [P(+) group, n = 9] and [P(−) group, n = 11]. Adiponectin levels rose significantly in both the P(+) and P(−) groups, and there were no differences between the groups. MCP-1 and ACR levels decreased significantly in both the P(+) and P(−) groups with no differences between the
### Table 3. Changes of adiponectin, MCP-1, IL-6, and urinary ACR

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sarpogrelate</th>
<th>Aspirin</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
</tbody>
</table>

<sup>a</sup>MCP-1, monocyte chemoattractant protein-1; ACR, urinary albumin-to-creatinine ratio.

<sup>b</sup>Sarpogrelate versus aspirin.

<sup>c</sup>P < 0.01, before versus after.

### Table 4. Differences of changes in any parameters with or without pioglitazone

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pioglitazone (+) (n = 9)</th>
<th>Pioglitazone (-) (n = 11)</th>
<th>p&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
</tbody>
</table>

<sup>a</sup>Pioglitazone (+) versus (−).

<sup>b</sup>P < 0.01, before versus after.

Two groups. We also compared the effect of aspirin between P(+) and P(−) groups. There were no differences in any of the parameters between the two groups at baseline; neither were there any changes from baseline to 16 wk of treatment with aspirin in either the P(+) or the P(−) group.

Figure 1 shows the correlation of percentage change of adi-
ponectin, MCP-1, and ACR in the sarpogrelate group. The percentage change of adiponectin correlated negatively to that of plasma MCP-1 levels (A), and the percentage changes of plasma and urinary MCP-1 correlated positively to that of change of ACR (B and C); however, the percentage change of adiponectin did not correlate with that of change of ACR (D).

Figure 2 depicts the relationship between percentage changes in ACR and percentage changes of either plasma MCP-1 or urinary MCP-1 in the whole study population. There were no significant relations.

Discussion
We and others previously showed that the 5-HT2A receptor antagonist sarpogrelate decreases ACR in patients with type 2 diabetes (2,25). In addition, sarpogrelate has been shown to increase plasma adiponectin levels in patients with ASO or diabetes (21,22); however, no previous study examined the relationship between changes in adiponectin and ACR in the action of sarpogrelate, and the mechanisms of the antialbuminuric action of sarpogrelate remains largely unknown. This study is the first to address these issues, by investigating several important factors involved in the pathophysiology of diabetic nephropathy. Our results clearly demonstrate that in patients with diabetic nephropathy and ASO, sarpogrelate not only increases plasma adiponectin levels but also decreases ACR and plasma and urinary MCP-1. The decreases of ACR were closely related to the decreases in plasma and urinary MCP-1. Aspirin, another antiplatelet drug, however, had no such effects. In this study, all of the patients had already been treated with renin-angiotensin system (RAS) inhibitors, yet sarpogrelate was still able to reduce ACR significantly. In addition, sarpogrelate could reduce ACR regardless of whether patients were treated with pioglitazone. Because reduction of ACR is closely related to improved prognosis in patients with diabetes, sarpogrelate may be a useful adjunctive medication to the currently established therapy that includes RAS inhibitors and TZD.

Recent studies have shown that in diabetic nephropathy, the plasma adiponectin level is positively associated with the amount of urinary albumin excretion, and it is particularly high in overt nephropathy (26). Adiponectin is thought to protect against vascular injuries; however, the sarpogrelate-induced increase in adiponectin may not be directly involved in the antialbuminuric mechanism of sarpogrelate, because there were no correlations between changes in plasma adiponectin levels and ACR.

The profile of the changes in plasma cytokines and inflammation markers induced by sarpogrelate is different from that previously observed with either RAS inhibitors or TZD. The administration of either ARB or pioglitazone is known to increase adiponectin and decrease not only MCP-1 but also IL-6 and hsCRP (16–19). We did not observe any changes in the level of IL-6 or hsCRP with sarpogrelate. In addition, all of the patients had already been treated with RAS inhibitors in this study; therefore, the actions of sarpogrelate that we observed do not seem to be mediated by the RAS. Neither is it likely that sarpogrelate acts via the same mechanisms by which TZD reduces ACR and increases adiponectin, because sarpogrelate’s effects were the same in the patients who were treated with or without pioglitazone.

The mechanisms by which sarpogrelate increases plasma adiponectin and decreases plasma MCP-1 remain largely unknown from this study; however, we speculate that sarpogrelate increases adiponectin production by inhibiting macrophages in the adipose tissues, because increases in adiponectin were significantly related to decreases in plasma MCP-1 levels. It has been shown that macrophages that accumulate in the adipose tissues cause a variety of biologic effects, such as suppression of adiponectin production (27–30); therefore, sarpogrelate-induced decreases in MCP-1 would be expected to
result in increases in adiponectin production by adipose tissues. AngII and 5-HT may increase MCP-1 in different pathways. In vascular smooth muscles, AngII increases oxidative stress (ROS), which activates the JAK/STAT-3 pathway, thus leading to increased MCP-1 (31); however, 5-HT activates the JAK2/STAT-1 pathway by way of the 5-HT2A receptor without the mediation of ROS (15). Thus, unlike ARB or ACEI, sarpogrelate may decrease MCP-1 by the pathway not involving ROS. A difference in pathways such as this may have been one of the reasons that MCP-1 was reduced by addition of sarpogrelate on top of ARB.

It has been reported that both plasma MCP-1 and urinary MCP-1 levels rise along with progression of nephropathy (8,9) and that a rise in MCP-1 correlates with renal tubular injuries (10–13). It is thought that macrophages and MCP-1 play an important role in renal damages of various causes (32). In diabetic nephropathy, a large number of macrophages accumulate in renal tubules, thereby causing local inflammations (7–14,32). It is postulated that urinary MCP-1 may reflect renal tubular inflammations. Because sarpogrelate-induced decreases in ACR and MCP-1 were significantly related each other, it may be speculated that sarpogrelate reduced renal tissue inflammations, at least in part, by inhibiting macrophage activation through reductions in MCP-1; however, we cannot rule out the possibility that MCP-1 was decreased secondary to reduced albuminuria as a result of a completely different mechanism. Indeed, when the sarpogrelate and aspirin groups were combined, there were no significant relationship between changes in ACR and changes in urinary or plasma MCP-1. Thus, in addition to reducing MCP-1, sarpogrelate may have some other unique mechanism of reducing ACR in patients with diabetic nephropathy.

In addition to inhibiting MCP-1 production, sarpogrelate may suppress macrophage activation by a different mechanism. In monocytes, serotonin enhances the expression of cholesterol acyltransferase-1 via the 5-HT2A receptor pathway, thereby increasing the intracellular accumulation of cholesterol ester (33). This would lead to foam formation of macrophages from monocytes. Sarpogrelate is reported to inhibit this pathway and suppress the intracellular accumulation of cholesterol in the monocytes (33); therefore, it may be possible that sarpogrelate may have decreased ACR, at least in part, by inhibiting lipid accumulations in the monocyte in the kidney. Further study is clearly needed to clarify the mechanisms.

**Limitations of the Trial**

This is a clinical study involving human subjects, so there is a limitation as to the degree to which the study was able to elucidate the mechanism of sarpogrelate-induced reduction of ACR. To inhibit platelet function, we used aspirin as a control drug, so we recruited only patients who had not been treated with aspirin. Aspirin is widely used in such patients who meet our inclusion criteria; therefore, we had a limited number of patients. In addition, there may be a selection bias; however, this would not affect the differences that we observed between the sarpogrelate and aspirin groups, because the patients were randomly assigned and there were no differences in the baseline clinical characteristics. Finally, sarpogrelate administration is indicated only for the patients with ASO in Japan; therefore, we included only patients with diabetic nephropathy and ASO. The issue of whether our results can be extended to patients with diabetic nephropathy in general awaits further investigation with large numbers of patients.

**Potential Clinical Implications**

The results of this study suggest the possibility that in patients with diabetic nephropathy and extensive vascular disorders, sarpogrelate can be expected to exert antialbuminuric actions that are based on different mechanisms than ARB or pioglitazone. In practice, many patients with diabetic nephropathy have severe vascular disorders in combination. In patients who are already given ARB and pioglitazone but still have clinically significant albuminuria, administration of sarpogrelate may have the potential to reduce urinary albumin excretion. Because changes in urinary albumin excretion have been shown to predict clinical outcome in patients with diabetes, administration of sarpogrelate may be considered a useful adjunctive therapeutic modality to the currently established treatments.

**Acknowledgments**

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**Disclosures**

None.

**References**

7. Furuta T, Saito T, Ootaka T, Soma J, Obara K, Abe K,


