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Protective Effect of Beraprost Sodium, a Stable Prostacyclin Analogue, on Cardiac Allograft Vasculopathy in Rats

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ABSTRACT

Intimal thickening and luminal narrowing of the coronary arteries are insidious complications of cardiac allograft. However, the pathogenesis of cardiac allograft vasculopathy (CAV) remains to be clarified. In this study, the protective effect of a prostacyclin analogue (beraprost sodium; BPS) on CAV was evaluated after heterotopic cardiac transplantation in rat. All recipients were treated with cyclosporine A (10 mg/kg/day intramuscularly). Eight rats received oral therapy with BPS of 50 μg/kg/day (BPS group) and another 8 rats received vehicle only (control group). All surviving cardiac grafts were removed on the 60th postoperative day and were examined to determine the severities of cellular rejection and CAV (>50 μm in diameter). Additionally, 6-keto-prostagrandin $F_{1\alpha}$ and thromboxane B_2 were compared between the two groups. There was no significant difference in the grading score for cellular rejection based on the ISHLT grading system (2.31 ± 0.75 vs 2.47 ± 0.65, p=0.81). Although the endothelial cells were preserved in both groups, a deposition of fibrin-like dense materials was recognized in the subendothelial layers of the control group, but not in the BPS group. Intimal thickening was inhibited significantly in the BPS group. The intimal ratio (intimal area/sectional area of artery) was significantly lower in the BPS group than in the control group (0.134 ± 0.03 vs 0.205 ± 0.047; p<0.01), without any difference in the medial ratio (medial area/sectional area of artery). α-actin positive smooth muscle cells (SMC) in intima were fewer in number in the BPS group than in the control group. The plasma thromboxane B_2 level was significantly lower in the BPS group than in the control group (270 ± 116 pg/ml vs 585 ± 258 pg/mg; p<0.01). It was concluded that BPS suppressed CAV development after heterotopic allogenic cardiac transplantation in rats.

Key words: *Cardiac allograft vasculopathy, Heart transplantation, Beraprost, Prostagrandin I_2*

As a result of advances in immunosuppression therapy, graft and patient survival rate has been improved in cardiac transplantation[5)]. However, intimal thickening and luminal narrowing of the coronary arteries, termed cardiac allograft vasculopathy (CAV), as well as grafted coronary arteriosclerosis (GCA), have remained insidious complications and the greatest limitations to long-term survival after heart transplantation[2,34)]. The mechanism of CAV is not fully understood, although it is suggested that it is immunologic and related to cytomegalovirus infection[11)] and hyperlipidemia[8,25)]. Regardless of the variety of potential therapies suggested by the multifactorial etiology of CAV[12,19)], a proven prophylaxis or treatment (other than retransplantation) has yet to be established.

Recently, it was reported that prostacyclin (PG I_2), which is the principal arachidonic acid metabolite synthesized mainly by the endothelium, plays an important role in the regulation of vascular homeostasis[21)]. PG I_2 and its stable analog increase the level of intracellular cyclic 3',5'-adenosine monophosphate (cAMP) via the activation of adenylate cyclase. These metabolites have various effects, including: potent antiplatelet and vasodilating activities[21)]; inhibition of the proliferation and migration of vascular smooth muscle cells (SMC)[22,33)]; inhibition of the release of interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) from monocytes[7,14,17)]; inhibition of neutrophil migration and of superoxide generation[26,38)]; suppression of the release of platelet-derived growth factor (PDGF)[35)], and a cytoprotective effect on vascular endothelial cells[30)]. Beraprost sodium (BPS) is a chemically stable PG I_2 analog which mimics PG I_2 in its biological properties. Moreover, BPS is the first

PG I_2 analog to become available for oral clinical administration and is known to have a long biological half life[39]. Therefore, BPS is expected to inhibit the development of CAV.

In this study, the protective effects of BPS on CAV were evaluated after heterotopic cardiac allogenic transplantation in rats.

MATERIALS AND METHODS

Preparation of drugs

Beraprost sodium (Kaken Pharmaceutical Co. Ltd., Urayasu, Japan) was diluted with distilled water to yield a final concentration of 10 μg/ml for oral administration. Cyclosporine A (Sandoz, Pharmaceuticals Ltd., Tokyo, Japan) was diluted to yield a final concentration of 10 mg/ml with Migryol 812 (Hüls Aktiengesellschaft, Marl, Germany) for intramuscular injection. These drugs were prepared within one week before use. Pentobarbital sodium (Nembutal™: Abbott Lab., Chicago, USA.) was diluted in isotonic saline to yield a final concentration of 10 mg/ml just before use for anesthesia.

Experimental animals

Male inbred Lewis (RT-1^l) rats (body weight of 214.6 ± 11.7 g, 8–10 weeks of age) and male inbred Fisher 344 (RT-1^{lvl}) rats (body weight of 209.9 ± 13.5 g, 8–10 weeks of age) were used as recipients and donors, respectively. All rats were supplied by the Charles-River Co. (Yokohama, Japan), and were maintained on the regular chow (MF: Oriental Yeast Co., Tokyo, Japan). Oral intake of water and food were allowed anytime. The temperature in the cage was maintained at 20–25°C with a 12–14 hr light cycle per day. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No.86–23, revised 1985).

Heterotopic heart transplantation

Every rat was anesthetized with a single intraperitoneal injection of 40 mg/kg of pentobarbital sodium. The cardiac graft from the donor rat was implanted in the neck of the recipient rat by anastomosis of the donor's pulmonary artery and brachiocepharic artery to the recipient's right jugular vein and left common carotid artery, respectively, in the manner described previously[18] according to the modified technique of Suzuki et al[36]. The ischemic time was 62.8 ± 3.7 min with no significant difference between each group. Every transplanted heart resumed beating well within 30 seconds after declamping and survived until the scheduled sacrifice.

Experimental design

All heart-transplanted recipients were treated with cyclosporine A, administered intramuscularly at a dose of 10 mg/kg/day for 20 days. Eight rats received compulsorily oral administration of beraprost sodium of 50 μg/kg/day (BPS group) and another 8 rats received vehicle only (control group). All surviving cardiac grafts and blood samples were harvested on the 60th postoperative day. The dose of cyclosporine A was determined on the basis of previously published information using the same rat combination[23]. The dose of BPS administered in this experiment was within the range of having antiplatelet effects[39] and below the level of causing subacute and chronic toxicity[1,24,29].

Light microscopic analysis

The transplanted hearts were fixed in 10% formalin solution for light microscopic observation. The hearts were then serially cut into 2 mm thick cross sections and embedded in paraffin. Tissue sections of 4 μm thickness were stained with hematoxylin & eosin (HE) and elastic van Gieson stain (EVG). For immunohistochemical observation, the tissue sections were immunostained using the abidin-biotin complex (ABC) method (VECTASTAIN Elite ABC-kit™, PK-6100, Vector Laboratories Inc., Calf., USA) with a monoclonal antibody against α-smooth muscle actin (dilution 1:100, DAKO Japan Co. Ltd., Tokyo, Japan). The sections were visualized with 3–3'diaminobenzidine (DAB). For additional staining of the elastic fibers, the sections were incubated in victoria blue solution (Muto Pure Chemicals Ltd., Tokyo, Japan) for 18 hours, and then counterstained with methyl green (Katayama Chemical, Osaka, Japan).

Electron microscopic analysis

Small tissue samples were obtained from the left ventricular wall of the transplanted hearts, fixed overnight in cold 2.5% glutaraldehyde solution buffered with phosphate at pH 7.4 and then postfixed for 1 hour in 1% OsO_4 solution buffered with phosphate at pH 7.4. Thereafter, the tissue samples were dehydrated through immersion in graded alcohol solutions and then embedded in epoxy resin. Semi-thin sections were cut with a Porter-Blum ultramicrotome equipped with glass knives, and the sections were stained with methylene blue (Richardson's solution). Thin sections were cut with the same ultramicrotome equipped with a diamond knife, and then stained with uranyl acetate and lead citrate, for observation with a transmission electron microscope at 75 kV (H-7000, Hitachi, Japan).

Grading of graft rejection and CAV

Using tissue sections stained with HE, the degree

Table 1. Score of histologic grades for cellular rejection[4)]

Score	Grade	Histologic appearance
0	0	No rejection
	I	Mild rejection (without myocyte damage)
1.0	A	focal and perivascular lymphocytic infiltrate
1.5	B	diffuse and interstitial lymphocytic infiltrate
2.0	II	Focal moderate rejection (with focal myocyte damage)
	III	Moderate rejection (with obvious myocyte damage)
3.0	A	multifocal and aggressive lymphocytic infiltrate
3.5	B	more diffuse and aggressive lymphocytic infiltrate, focal hemorrhage,
4.0	IV	Severe rejection (with obvious myocyte necrosis) severe lymphocytic infiltrate, diffuse hemorrhage

of graft cellular rejection was graded from 0 to 4 according to the standardized cardiac grading system recommended by the International Society for Heart and Lung Transplantation (ISHLT)[4)], as summarized in Table 1. Evaluation was carried out blindly by two observers. For analysis of the degree of intimal thickening, the cross sectional area of the intimal layer, medial layer, and lumen were measured in intramyocardial coronary arteries larger than 50 μm in diameter. Measurements were repeated twice blindly by a single observer by means of a Macintosh computer with analytical software (Canvas™, Deneba Systems Inc., USA). Tangentially cut vessels were excluded from the measurement in order to minimize the error in estimating intimal thickening. The intima to media ratio (I/M ratio), luminal narrowing (LN), intimal ratio and medial ratio were calculated with the following formula:

I/M ratio=(Intimal area)/(Medial area)

LN=(Intimal area)/(Intimal area+Luminal area)

Intimal ratio=(Intimal area)/(Cross sectional area)

Medial ratio=(Medial area)/(Cross sectional area)

Proliferation of SMC in the intima was quantified using the intimal SMC index, which was calculated as follows.

Intimal SMC index=N / A

N: the number of cells in the intima which were positive for α-smooth muscle actin

A: the area around the internal elastic lamina of the coronary artery

Biochemical analysis

After the transplanted heart had been harvested, a blood sample was taken from the abdominal aorta using a 19 gauge needle. The following parameters were measured: serum total cholesterol (T-chol), serum triglyceride (TG), plasma thromboxane B_2 (TX B_2) as an indicator of thromboxane A_2 (TX A_2), and plasma 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PG $F_{1\alpha}$) as an indicator of plasma PG I_2. Serum concentrations of T-chol and TG were determined using standard enzymatic packages (Wako Pure Chemical Co., Osaka, Japan). Plasma TX B_2 and 6-keto-PG $F_{1\alpha}$ concentrations were determined using radioimmunoassay kits (DuPont NEN Research Products, USA).

Statistics

All values were expressed as mean ± one standard deviation (SD). Differences in the grade of cardiac cellular rejection were compared using the Mann-Whitney U test. Differences in the nominal values were evaluated using the unpaired Students' t test. Differences were considered to be statistically significant if the p value was less than 0.05.

RESULTS

Light microscopic analysis

A. Degree of cellular rejection

In the myocardium of the transplanted heart of both the control and BPS groups, focal inflammatory infiltrations, variable in size and shape, were scattered throughout the myocardium with myocyte replacement. According to the standardized cardiac grading system for cellular rejection (by ISHLT), almost all of the grafted hearts showed grade II to III rejection (mean rejection score: control 2.31 ± 0.75, BPS 2.47 ± 0.65) (Table 2). Quilty effect-like focal accumulations of lymphocytes, which were predominantly confined to the endocardium, were present as illustrated in Fig. 1. In the epicardium of both groups, severe inflammatory cell infiltration with fibrosis was commonly observed. This finding was considered to be the result of non-specific inflammation following

Table 2. Morphometric parameters reflecting graft rejection and allograft coronary disease

	Control group	BPS group	p value
Rejection score	2.31 ± 0.75	2.47 ± 0.65	N.S.
Number of vessels	46.1 ± 7.9	40.3 ± 7.2	N.S.
I/M ratio	0.402 ± 0.102	0.277 ± 0.079	$p < 0.05$
Luminal narrowing	0.457 ± 0.076	0.275 ± 0.04	$p < 0.01$
Intimal ratio	0.205 ± 0.047	0.134 ± 0.03	$p < 0.01$
Medial ratio	0.563 ± 0.052	0.564 ± 0.053	N.S.
Intimal SMC index	51.5 ± 15.8	37.8 ± 8.2	$p < 0.05$

Each value is the mean ± SD. Rejection score was tested by Mann-Whitney U test. Others were tested by unpaired Students' t test.
Statistical significance is $p<0.05$. N.S.: not significant.
I/M ratio: intima to media ratio. SMC: smooth muscle cell.

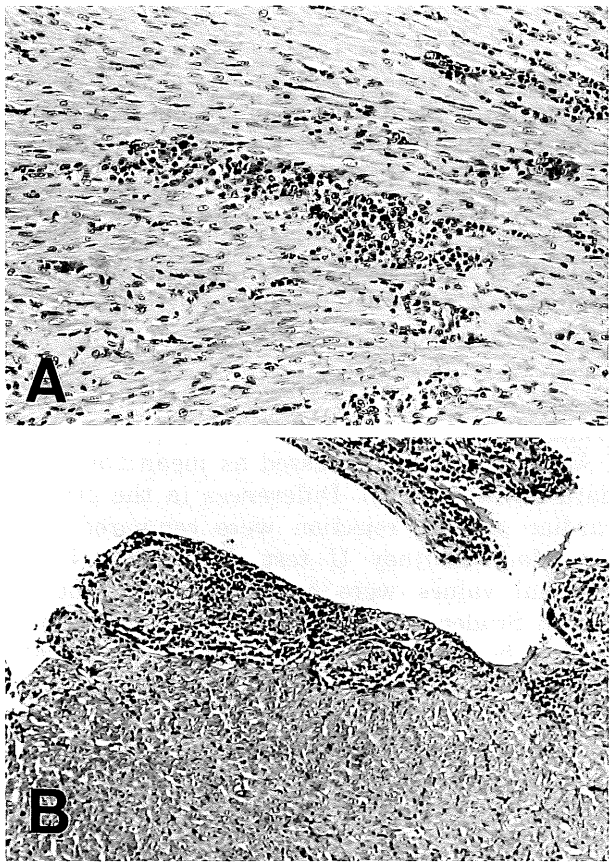

Fig. 1. A: Representative lesion of moderate cellular rejection (grade IIIA). There are focal lymphocyte infiltrations with obvious myocyte damage and myocyte replacement (hematoxylin & eosin, ×200). **B:** Focal accumulations of lymphocytes confined predominantly to the endocardium. The lesions, which were similar to the so-called "Quilty effect" of human cardiac transplantation, were observed in both control and BPS groups (hematoxylin & eosin, ×170).

transplantation. No significant differences were found in the histologic severity of graft cellular rejection between the groups (p=0.81)

B. Changes in the coronary arteries

There was no difference in the total number of cross sectional coronary arteries (<50 μm in diameter) per heart between the control group and the BPS group (46.1 ± 7.9 and 40.3 ± 7.2, respectively). Various changes in the coronary arteries were observed in both groups. Most of the coronary arteries showed evidence of concentric proliferation of the intima with almost an intact internal elastic lamina. The thickened intima consisted of spindle cell proliferation and fibrosis. The cells in the intima were positive for α-smooth muscle actin. Proliferation of elastic fibers (thin or thick), the so-called "intimal elastosis", was often observed in the thickened intima. However, the severe intimal thickening was not associated with intimal elastosis in some of the coronary arteries. Intimal elastosis appeared to be more severe in the control group than in the BPS group, although an appropriate grading scale for quantitative comparison was not available. The results of morphometric analyses of CAV are listed in Table 2. Typical examples are shown in Fig. 2. The I/M ratio and LN in the BPS group were significantly lower than in the control group (0.277 ± 0.079 vs 0.402 ± 0.102, $p<0.05$; 0.275 ± 0.040 vs 0.457 ± 0.076, $p<0.01$, respectively). The ratio of intimal area to cross sectional area in the BPS group was significantly lower than in the control group (0.134 ± 0.03 vs 0.205 ± 0.047, $p<0.01$). However, no significant difference was detected between the ratio of medial area to cross sectional area of the two groups (BPS group: 0.564 ± 0.053 vs control group: 0.563 ± 0.052, p=0.96). The intimal SMC index was lower in the BPS group than in the control group (37.8 ± 8.2 $\times 10^2/mm^2$ vs 51.5 ± 15.8 $\times 10^2/mm^2$, $p<0.05$).

Transmission electron microscopy

The endothelial cells of the coronary arteries were well preserved in both groups. Deposition of fibrin-like dense materials was, however, fre-

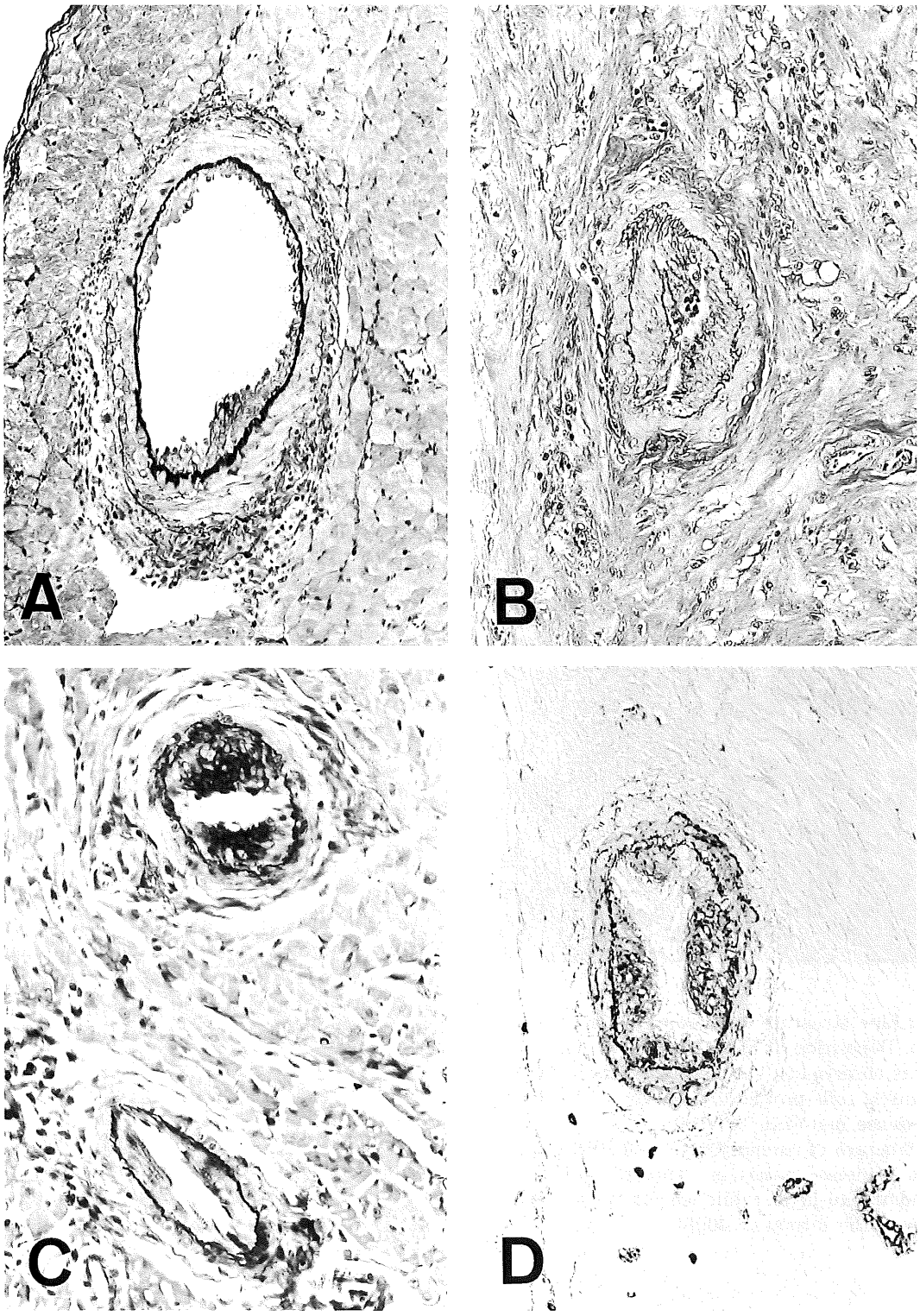

Fig. 2. Intramyocardial coronary arterial changes. **A:** Internal elastic lamina is almost intact. Mild thickening of coronary arterial intima (elastic van Gieson, ×220). **B:** Marked thickening of coronary arterial intima. Concentric narrowing of arterial lumina with intimal cell proliferation and fibrosis is distinct. But internal elastic lamina is well preserved (elastic van Gieson, ×200). **C:** Severe intimal elastosis. Densely proliferated elastic fibers are observed in intima (elastic van Gieson, ×380). **D:** Moderate intimal thickening of coronary artery. Proliferated cells of intima are positive for α-smooth muscle actin (victoria blue and α-smooth muscle actin, ×220).

quently observed in the subendothelial layer of the coronary arteries in the control group (Fig. 3A). Moreover, focal or diffuse proliferation of SMC was usually detected between the endothelial cells and the dense material. A small number of collagen fibers appeared around the prolifer-

ated SMC, although fibroblasts were absent in the subendothelial layer. In the BPS group, proliferation of SMC was also observed in the subendothelial layer. However, the deposition of dense materials was difficult to detect in the subendothelial layer of the coronary arteries (Fig. 3B).

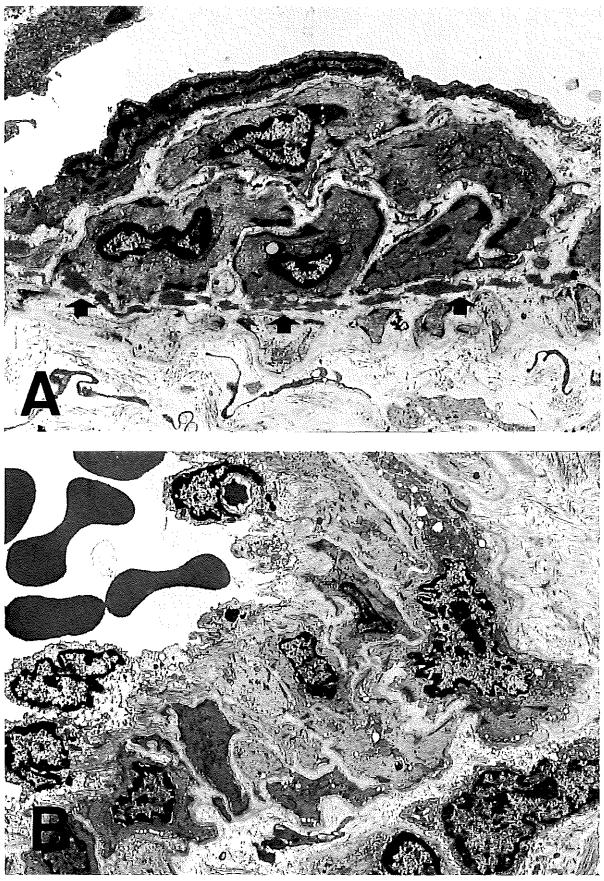

Fig. 3. A: Fine structure of coronary artery of control group. Deposition of fibrin-like dense materials (arrow) was observed in subendothelial layer. Many smooth muscle cells proliferate between endothelial cells and dense materials (×5100).
B: Fine structure of coronary artery of BPS group. Deposition of dense materials is not recognized in the subendothelial layer, while smooth muscle cells proliferate in the intima (×4000).

Biochemical analysis

As shown in Table 3, there was no statistical difference in T-chol between the two groups (BPS group: 49.1 ± 8.95 mg/dl vs 51.7 ± 4.13 mg/dl, p=0.46). The triglyceride concentration in the BPS group was significantly lower (26.0 ± 17.2 mg/dl vs 59.4 ± 29.9 mg/dl, $p<0.05$), but TG levels in both groups were not higher than the normal range. The 6-keto-PG $F_{1\alpha}$ concentration in the BPS group (181 ± 145 pg/ml) was not statistically different from that in the control group (215 ± 155 pg/ml). The TX B_2 concentration in the control group (585 ± 258 pg/ml) was significantly higher than that in the BPS group (227 ± 116 pg/ml). Thus, the TX B_2/6-keto-PG $F_{1\alpha}$ ratio tended to be higher in the control group than in the BPS group (3.9 ± 2.4 vs 1.8 ± 1.1, p=0.056).

DISCUSSION

The present study demonstrated that the oral administration of beraprost sodium inhibits the development of CAV in the cyclosporine-treated rat model.

In the allogenically grafted hearts of long-term human survivors, CAV is most commonly characterized by coronary arterial changes, including intimal thickening and concentric narrowing of the vascular lumina with nearly intact internal elastic lamina[19]. The precise mechanism of these changes is still unknown. Some investigators have suggested that CAV may be the result of intimal injury associated with an immune response[28].

In the present study, changes in the coronary arteries similar to those in human cases developed in a rat allograft model using cyclosporine A 10 mg/kg/day for 20 days, as previously described by Nagamine[23]. The intima of the intramyocardial coronary arteries was thickened to variable degrees with a proliferation of spindle cells and elastic fibers. These intimal spindle cells were SMC because they were immunohistochemically stained with α-smooth muscle actin. In electron microscopic observations, the proliferated intimal cells were identified as SMC, which

Table 3. Biochemical analysis of serum lipid and prostanoids

	Control Group	BPS Group	p value
Total cholesterol (mg/dl)	51.7±4.1	49.1±8.9	N.S.
Triglyceride (mg/dl)	59.4±29.9	26.0±17.2	$p < 0.05$
6ketoPG $F_{1\alpha}$ (pg/dl)	215±155	182±145	N.S.
Thromboxane B_2 (pg/dl)	585±258	270±116	$p < 0.01$
TX B_2/6ketoPG $F_{1\alpha}$	3.9±2.4	1.8±1.1	N.S.

Values are mean±SD. Comparisons were tested by unpaired t test.
N.S.: not significant. PG: prostagrandin. TX: thromboxane.
BPS: beraprost sodium.

contained numerous fine filaments attached to dense patches. These findings in the present study supported the observations of Ito et al[13] who attributed the intimal thickening to the proliferation of SMC.

BPS significantly inhibited the intimal thickening, resulting in a significant reduction of luminal narrowing, without any changes of the grade of cellular rejection. BPS suppressed the proliferation of SMC in the intima, as shown by the intimal SMC index. In the electron microscopic analysis, fibrin-like dense material, as well as SMC, was frequently observed in the subendothelial layer in the control group, but only scarcely in the BPS group. Although the fine structures of the vascular endothelial cells were well preserved at the time of sacrifice in both the control and BPS groups, the deposition of dense material suggests that the endothelial cells were damaged more or less after cardiac transplantation and that plasma proteins leaked through the endothelial cells. These fine structural changes suggest that the leakage of the plasma protein was larger in the control group than in the BPS group. Variable numbers of elastic fibers, indicating elastosis, appeared in the intima with fibrous thickening. Although the pathogenesis and functional significance of intimal elastosis remains unclear, the proliferation of elastic fibers was less severe in the BPS group than in the control group. These findings indicate that BPS significantly inhibits the production of elastic fibers.

Some investigators have demonstrated that the development of CAV is immunological in origin and is mediated by multiple steps at different periods[3,6,10]. For instance, endothelial injury acting as a trigger, activated macrophage infiltration in the early period, and later SMC proliferation all play important roles in the development of CAV. Although PG I_2 and its analogue have various suppressive effects on a broad range of cellular activity[7,21,26,30,33,38], there was no significant difference in the histologic severity of graft cellular rejection between the two groups. Therefore, the prevention of intimal thickening cannot be attributed to enhanced suppression of the cellular immune response by BPS. BPS appears to suppress the CAV during two steps: first, during endothelial injury, and second, during SMC proliferation. Sakai et al[30] demonstrated the cytoprotective effect of BPS against chemical injury in cultured human vascular endothelial cells and suggested that it could be derived from the increase in the cAMP level in endothelial cells via the activation of adenylate cyclase in cultured endothelial cells. In this study, deposition of dense materials suggested that the endothelial cells were more highly damaged in the control group than in the BPS group. As to the effects of BPS on the proliferation of SMC, an indirect effect of BPS may be suppression of the proliferation of SMC by inhibiting the release of PDGF via antiplatelet effect[35], while PDGF has been reported to play an important role in CAV[9]. BPS may directly suppress the DNA synthesis in SMC by acting on the progression stage of the G_1 phase in the cell cycle[22,33] and the phenotype change of SMC by increasing intracellular cAMP[27]. However, the effect of BPS on the cell cycle and phenotype change of SMC will need to be further investigated.

BPS also significantly diminished plasma TX B_2 concentration. From the viewpoint of regulation of vascular homeostasis, eicosanoids, particularly PG I_2 and TX A_2, are considered to function in the maintenance of "vascular integrity"[21]. Sicard et al[32] concluded that the balance between TX A_2 and PG I_2 production in the grafted vascular wall might be the best determinant of the long-term patency of small-caliber grafts. In this study, the plasma TX B_2 level was significantly lower in the BPS group after transplantation than in the control group. Consequently, BPS may have the effect of improving the balance between TX A_2 and PG I_2 production. It is considered that an increase of cAMP level in the BPS group may result in suppression of TX A_2 production, although the diminution of PG I_2 due to endothelial injury after transplantation stimulates platelets to produce TX A_2. Kouchi et al[15] demonstrated that both TX B_2 and TX B_2/6-keto-PG $F_{1\alpha}$ were decreased after administration of BPS, and concluded that BPS might prevent intimal hyperplasia in the peripheral arterial grafting model. Teraoka et al[37] showed that thromboxane synthetase inhibitor has a suppressive effect on chronic vascular rejection after kidney transplantation. Thus, one of the suppressive effects of BPS on CAV may be the prevention of TX A_2 production.

Several investigators have reported that hypercholesterolemia accelerated human CAV[2,4,25]. Oral therapies of fish oil[31,40] and of sinvastatin[20] significantly inhibited CAV in the experimental hypercholesterolemia model. As regards the influence of PG I_2 on cholesterol metabolism, PG I_2 stimulates activity of both lysosomal (acid) cholesteryl ester hydrolase and cytoplasmic neutral cholesteryl ester hydrolase by cAMP and cAMP-dependent protein kinase, respectively, in intact arterial SMC[27]. BPS has also been reported to decrease the serum cholesterol level in cholesterol-fed rabbit[16]. In this model with a standard diet, hyperlipidemia and changes of T-chol level were not observed in either group. Only the triglyceride level was lower in the BPS group than in the control group. Further investigation is necessary to clarify the effect of a lower triglyceride level on protecting CAV, although cholesterol metabolism can likely be excluded as

a possible mechanism contributing to the inhibitory effect of BPS on CAV.

Several limitations of this study must be acknowledged. The metabolism of arachidonic acid and the lipid metabolism and pharmacokinetic characteristics of BPS are likely to be different among animals. Moreover, heterotopic, non-working cardiac allografts may be different from a working orthotopic transplant model in terms of the vascular disease and therapeutic intervention. The potential usefulness of BPS for suppressing CAV, as demonstrated in this study, cannot thus be directly extrapolated to humans.

In summary, ultrastructural, immunohistochemical and biochemical analyses demonstrated that BPS significantly inhibits the development of CAV by several possible mechanisms: 1) protection of endothelial cells; 2) inhibition of SMC proliferation; and 3) maintenance of vascular homeostasis by regulation of the eicosanoid cascade. Further investigations will be needed to clarify the mechanism of the protective effect of BPS in greater detail.

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