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Ketanserin Reduces Graft Arteriosclerosis After Allogeneic Aorta Transplantation in Rats

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Abstract

Summary: The serotonin-2 receptor antagonist ketanserin has been suggested to diminish arteriosclerotic development by its effect on platelet function and on vascular smooth muscle cells. We investigated the ability of ketanserin in reducing immune-mediated arteriosclerosis using the BN-WAG and WAG-BN rat aortic transplantation models. Ketanserin (10 mg/kg/day) administered in drinking water significantly reduced posttransplant arteriosclerotic thickening of the intima in the BN-WAG rat model to 102 ± 23 μm as compared with 171 ± 60 μm in untreated BN-WAG allografts 8 weeks posttransplantation ($p < 0.05$). In the opposite WAG-BN combination, at 4 weeks posttransplantation, no significant reduction in intimal thickening was attained (112 ± 42 vs. 152 ± 49 μm). Platelet aggregation to increasing amounts of collagen did not show a correlation between the effect of ketanserin on platelet function and reduction in intimal thickening. Ketanserin had no effect on systolic blood pressure or mononuclear cell infiltration. We conclude that ketanserin reduces graft arteriosclerosis by a mechanism other than by inhibition of platelet function, decrease in blood pressure, or immunosuppression. Because of this antiarteriosclerotic effect, ketanserin therapy might be beneficial to the long-term survival of vascular allografts.

With decreasing numbers of acute graft loss, chronic rejection has become the major complication of clinical transplantation. In heart grafts, chronic rejection is dominated by concentric intimal thickening of the epicardial as well as the intramyocardial branches of the coronary arteries. Because of this localization pattern, this phenomenon is also termed graft vascular disease (GVD). The prevalence of GVD ranges from 2 to 18% at 1 year and from 50 to 73% at 5 years after transplantation (1-4). This narrowing of the coronary arteries is the major limiting factor in medium- and long-term graft survival after heart transplantation. That GVD is a major problem even during the first year after transplantation, resulting in 33 and 68% of patient death, has been reported by centers with long experience in heart transplantation (5,6). There is no current treatment for chronic rejection, and in patients with extensive GVD retransplantation remains the only option to prevent death (7).

The pathogenesis of graft arteriosclerosis has not yet been elucidated, but immunologic phenomena linked to histoincompatibility differences between donor and recipient is generally believed to result in the development of proliferative lesions (8,9). Repetitive endothelial injury with consequent platelet activation may thus contribute to the development of GVD (10,11).

Studies of the mechanism of classic atherosclerosis have generated a resurgence of interest in serotonin. Serotonin is stored in the dense granules of platelets and, when released, theoretically may stimulate thickening of the blood

Outline

vessel wall in several ways, first in the positive feedback that it exerts on platelet aggregation. By amplifying the release of other aggregatory stimuli, serotonin will facilitate ongoing platelet aggregation and thus the release of growth factors, in particular platelet-derived growth factor (PDGF) (12,13). Second, serotonin has been demonstrated to stimulate directly the mitogenesis of aortic vascular smooth muscle cells (VSMC) in culture. Although substantially less potent in this respect than PDGF, serotonin in low concentrations significantly potentiates the VSMC mitogenesis of PDGF (14). Last, serotonin may stimulate arteriosclerosis by its conceptive role in chronic hypertension (15). Indeed, blocking the serotonin-2 ($5\text{-}HT_2$) receptors by ketanserin, clinically applied as an antihypertensive agent, effectively antagonizes these effects of serotonin (16-18). Therefore, ketanserin has been suggested to provide protection to arteriosclerotic diseases (19).

In previous immunohistologic studies of graft arteriosclerosis in aortic allografts, we observed pre-dominantly actin-positive VSMC in late intimal lesions. Furthermore, we observed more severe arteriosclerotic alterations in the WAG-Brown Norway (BN) rat strain combination than in the reverse combination (20). Because of our results showing that the BN has a stronger blood-clotting tendency than the WAG rat, in the present study we examined the role of platelet aggregation in the development of posttransplant arteriosclerotic lesions. We also tested the hypothesis that ketanserin provides vascular protection by affecting platelet function and by blocking directly the growth stimulation of VSMC by serotonin. The effects of ketanserin on blood pressure and on collagen induced platelet aggregation were monitored.

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METHODS

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Experimental animals

Male inbred BN ($RT1^n$) and WAG ($RT1^u$) rats were used. All animals were obtained from Harlan CPB, Austerlitz, The Netherlands, and had free access to food and water. Rats of both strains, weighing 200-250 g and aged 10-12 weeks, were used as recipient and donor. The experimental protocols were approved by the Committee on Animal Research of Erasmus University, Rotterdam and adhered to the *Guidelines on the Protection of Experimental Animals* of the Council of the EC (1986).

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Aorta transplantation

All rats were anesthetized with ether and underwent laparotomy. In the donor as well as the recipient, a segment of infrarenal aorta, [almost equal to]1 cm long, was isolated, excised, perfused with saline, and used as a transplant. Donor aorta was transplanted into orthotopic position. End-to-end anastomosis was performed with a 9.0 monofilament nylon suture (Eticon, Sommerville, NJ, U.S.A.). The ischemic time was 20-30 min.

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Ketanserin treatment

Ketanserin (Ketanserin-tartrate, Janssen, Beerse, Belgium) was given orally in a daily dose of 10 mg/kg, dissolved in drinking water. Although this dose of ketanserin is high as compared with human standards, no toxic side effects were noted in a pilot study. Ketanserin was dissolved in drinking water to maintain constant blood level. To prevent precipitation, a freshly prepared ketanserin solution was provided daily. We ascertained intake of 10 mg/kg ketanserin by each animal by measuring their daily water consumption.

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Experimental design

In a previous study, we observed an increase in intimal thickness in the BN-WAG and WAG-BN aortic transplantation models as well as in BN and WAG aortic autotransplants (20). In the WAG-BN model, the process of intimal thickening was so rapid that severe lesions had already developed 4 weeks posttransplantation. Due to intense intimal thickening, thrombosis and necrosis, all allografts of this strain combination removed at later timepoints were not useful for evaluation. In the reverse combination (from the BN-WAG), intimal lesions developed more slowly. In this rat combination, a steady increase to severe lesions at 8 weeks was evident. Therefore, in this study, animals in the WAG-BN combination were killed at 4 weeks and those in the BN-WAG combination were killed at 8 weeks. The experiment was performed with four groups of rats. The different groups with corresponding treatments and numbers of the animals are shown in Table 1.

Table 1

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Histology

Straight 5-μm cross-sections from tissues embedded in paraffin were prepared at three levels of the midportion of the graft and stained with hematoxylin-eosin and with elastic of Gieson. Slides were then examined by light microscopy. The thickness was measured with a calibrated ocular micrometer to evaluate the following variables: the intimal and medial thickness, adventitial infiltration, and SMC necrosis.

The average medial and thicknesses and maximal intimal thickness were determined. Cellularity of the adventitia, as a measure of cellular infiltration, and of the media, was assessed by counting the number of nuclei at five sites. The mean score was multiplied to a field of 0.1 mm^2.

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In vitro platelet aggregation assays

Platelet function was measured at the times the animals were killed: at 4 weeks posttransplantation in the WAG-BN model and at 8 weeks in the BN-WAG model. Blood from all animals receiving ketanserin and blood from 6 untreated transplanted BN and WAG rats each, as controls, were analyzed. In addition, platelet function of 3 BN and 3 WAG rats was tested 3 h after administration of a high dose of ketanserin by gastric intubation (50 mg/kg) to determine whether the dose of 10 mg/kg reduced platelet aggregation in the experimental animals. Under ether anesthesia, the animals received 50 IU heparin intravenously, after which the abdomen was opened. At the time of death, the abdominal aorta was dissected at its bifurcation, and blood was collected by aortic puncture. Platelets were aggregated with the Chronolog-Whole Blood Aggrometer (Chronolog U.K.). Collagen was chosen to induce platelet aggregation because of its likely physiological role in mediating platelet aggregation in vascular allografts. Increasing concentrations of 0.025-2.50 mg/ml collagen were added to heparinized (10 U/ml) whole blood samples, which were diluted 1:1 with 0.9% saline. Ten-minute recordings of the induced changes in electrical resistance (impedance, in [OMEGA]) were analyzed by an Olivetti M24 PC. The amount of platelets was counted on a TOA platelet counter PL100.

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Measurement of blood pressure

The effect of ketanserin on systolic blood pressure was measured in conscious rats by the tail-cuff method with an electrosphygmomanometer (Narco Bio-Systems, Houston, TX, U.S.A.). Measurements were made in both BN-WAG and WAG-BN transplanted rats receiving ketanserin as well as in the untreated transplanted control rats 1 week before and 0.5, 1, and 2 weeks after transplantation.

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Statistical analyses

The results are mean and SD. The data were assessed statistically by analysis of variance (ANOVA) followed by a Bonferroni *t* test; $p < 0.05$ was considered statistically significant.

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RESULTS

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Effect of ketanserin on graft arteriosclerosis

As shown in Table 1, ketanserin significantly reduced intimal thickening in the BN-WAG combination to 102 ± 23 μm as compared with 171 ± 60 μm in the allogeneic controls 8 weeks after transplantation ($p < 0.05$). In the WAG-BN combination, no significant inhibition was observed 4 weeks after transplantation (121 ± 64 vs. 152 ± 49 μm). Ketanserin treatment did not affect adventitial cellular infiltration or SMC necrosis. No toxic side effects of ketanserin were noted during the experimental period.

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Platelet aggregation

There was no difference in the number of platelets between the BN and WAG rat strains. Changes in electrical resistance as a result of collagen-induced platelet aggregation in the different experimental groups are shown in Figs. 1-3. Untreated BN platelets were more sensitive to collagen than WAG platelets. Collagen in a concentration as low as 0.050 mg/ml was effective in inducing BN platelet aggregation. A concentration of 0.200 mg/ml decreased electrical resistance by 1.57 [OMEGA], whereas untreated WAG platelets were not affected at this


Fig. 1


Fig. 2

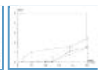
Fig. 3

concentration. Aggregation of WAG platelets was observed only at collagen concentrations >=0.250 mg/ml. After ketanserin treatment, BN platelet aggregation was observed at collagen concentrations >=200 mg/ml, showing an aggregation pattern similar to that of untreated WAG platelets. WAG platelet function at low collagen concentrations was not altered by ketanserin treatment. At high concentrations of collagen, no difference in electric resistance was observed in either rat strains as compared with the untreated transplanted controls. Similar patterns of collagen-induced platelet aggregation were noted 3 h after high-dose ketanserin (50 mg/kg) administration to normal rats of the two different strains (Fig. 3). This finding supports a maximal therapeutic effect of 10 mg/kg ketanserin as administered to the experimental animals.

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Effect of ketanserin on systolic blood pressure

Before transplantation, BN rats had systolic blood pressure of 125 ± 6 mm Hg as compared with 114 ± 7 mm Hg in untransplanted WAG rats. After BN-WAG and WAG-BN aortic transplantation, systolic blood pressure did not change in untreated controls (115 ± 7 and 121 ± 7 mm Hg at day 7 and 117 ± 8 and 126 ± 9 mm Hg at day 14 posttransplantation, respectively) or after ketanserin treatment (114 ± 7 and 128 ± 9 mm Hg at day 7 and 116 ± 9 and 132 ± 8 mm Hg at day 14).

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DISCUSSION

Ketanserin has been suggested to provide vascular protection by blocking the effects of serotonin on platelets and SMC (19). Indeed, in the present study, the development of posttransplant arteriosclerosis was significantly reduced in the BN-WAG combination. The ability of ketanserin to block 5-HT_2 receptors therefore suggests that platelet-derived serotonin might be involved in the mechanism leading to posttransplant arteriosclerosis. Serotonin-mediated platelet aggregation has been demonstrated to be reduced by ketanserin (16,17). In the present study, the sensitivity of BN platelets was also reduced to low concentrations of collagen after ketanserin treatment. The inhibiting effect of ketanserin on BN platelet aggregation did not coincide with significant inhibition of the arteriosclerotic response in the WAG-BN aorta transplantation model, however. Similarly, in isografts, a greater sensitivity of BN platelets did not result in more pronounced arteriosclerotic lesions in BN rats than in WAG rats (20). On the other hand, intimal thickening was significantly reduced in the BN-WAG model, in which no effect of ketanserin on collagen-induced platelet aggregation could be detected. Therefore, we noted no correlation between the effect of ketanserin on platelet function and reduction of intimal thickening, which suggests that ketanserin might reduce arteriosclerosis by a mechanism other than by affecting platelet function.

A direct blocking effect by ketanserin on the serotonin-induced mitogenesis of VSMC is a likely explanation for the antiproliferative effect. In vitro, serotonin has been reported to stimulate the mitogenesis of aortic VSMC (14). Any immunosuppressive effect of ketanserin is unlikely because no differences in mononuclear cell infiltration were evident between ketanserin-treated and untreated allografts in the present study.

The significant reduction in intimal proliferation induced by ketanserin in the BN-WAG aortic model suggests that the stimulating effects of platelet-derived serotonin on the mitogenesis of VSMC might be of relatively greater importance in the BN-WAG model than in the WAG-BN model, possibly owing to differences in mononuclear cell infiltration in the two rat strain combinations (20). More severe cellular rejection in the WAG-BN combination may indicate a minor role of serotonin in the overall arteriosclerotic stimulation after aorta transplantation. On the contrary, relatively weaker rejection in the BN-WAG combination may provide local conditions that cause serotonin to be of significant importance in stimulating VSMC proliferation.

Ketanserin did not affect blood pressure during the experiment. This finding is in agreement with results of previous studies that showed a minor and brief response of blood pressure to ketanserin in conscious normotensive animals as compared with anesthetized animals or conscious hypertensive rats (21,22). The hypotensive effect of ketanserin therefore can be excluded as a mechanism leading to decreased graft arteriosclerosis.

Serotonin has been suggested to be a mediator of cyclic flow alterations in stenosed coronary arteries in dog studies. These flow variations are believed to be produced by platelet aggregation at the stenotic site alternating with thrombus dislodgement and embolization. The frequency and severity of cyclic flow alterations and platelet aggregation predict the severity of neointimal

proliferation. Ketanserin treatment was successful in abolishing these blood flow variations and in retarding neointimal proliferation (23,24). Our study showed a decrease in BN platelet sensitivity to low concentrations of collagen with no effect on maximal platelet aggregation. Therefore, an increase in the threshold of platelet activation by ketanserin treatment might explain the mechanism of alternating cyclic flow variations (24). This hypothesis is also in accord with the reported effect of ketanserin in prolonging rat tail bleeding time (25).

Used clinically, ketanserin causes a greater decrease in blood pressure in hypertensive or older patients (21,22,26). Similarly, ketanserin causes a considerably greater reduction in serotonin-induced platelet aggregation in older than in younger patients (27). These findings suggest that ketanserin might be more effective in reducing posttransplant arteriosclerosis when serotonin is of greater importance in the associated hemodynamic processes. A similar explanation was offered in a large clinical study of restenosis after coronary angioplasty in which no protective effect by ketanserin was detected. The lack of effect in this trial may indicate a minor role of serotonin in the complex biology of late restenosis (28). Future studies of ketanserin in older, hypertensive, or partially immunosuppressed animals may demonstrate its effects in reducing posttransplant arteriosclerosis.

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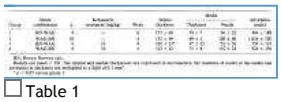

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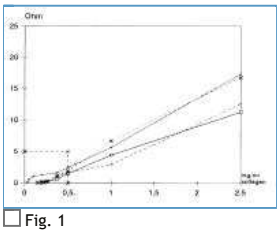
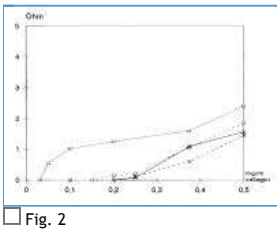


Fig. 1



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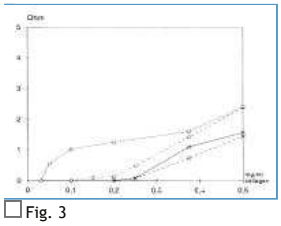

Fig. 3

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