Additive and Synergistic Effects of a Low-Molecular-Weight, Heparin-like Molecule and Low Doses of Cyclosporin in Preventing Arterial Graft Rejection in Rats

Didier Plissonnier, Gisèle Amichot, Josette Lecagneux, Micheline Duriez, Danielle Gentric, and Jean-Baptiste Michel

Arteriosclerotic intimal proliferation is one of the main long-term complications of organ transplantation. Low-molecular-weight, heparin-like molecules prevent myointimal proliferation in arterial wall injury and limit rejection in skin allografts. Cyclosporin limits rejection but has no major effect on intimal proliferation. Therefore, an experimental protocol was designed to test whether heparin-like molecules interacted with low doses of cyclosporin to prevent arterial wall immune system injury and response in a model of arterial graft rejection in normotensive and hypertensive rats. Aortic allografts were performed in spontaneously hypertensive rats (SHRs) and Wistar-Kyoto (WKY) normotensive control rats. Four groups of 10 allografted (SHR and WKY) rats were used: one group was treated with placebo, one with low doses of cyclosporin (2 mg/kg body wt per day), one with low-molecular-weight, heparin-like molecule (1 mg/kg body wt per hour), and one with low doses of cyclosporin plus low-molecular-weight, heparin-like molecule. Ten SHRs and 10 WKYs were isografted and served as the control groups. All rats were killed 8 weeks after aortic grafting. Structural parameters of the grafted segment were measured by morphometric analysis on formalin-fixed sections with specific stains. The classical signs of immune system injury and response were present in the untreated allografts in SHRs and WKYs: inflammatory infiltration of the adventitia, medial injury, and intimal proliferative response. Low doses of cyclosporin had a significant beneficial effect on immune medial injury by increasing medial thickness and the number of remaining smooth muscle cells and decreasing the extracellular matrix injury. Cyclosporin had no protective effect on intimal proliferation. Low-molecular-weight, heparin-like molecules had a beneficial effect on both the medial injury and the intimal proliferative response that was independent of blood pressure. Heparin-like molecules increased medial thickness and partially prevented smooth muscle cell loss and extracellular matrix attack. They also significantly decreased the intimal thickness by acting more on the collagen content than on the smooth muscle cell density. Low doses of cyclosporin plus heparin-like molecules had a marked effect in preventing the arterial wall injury and response. This combination resulted in a normal medial appearance, increased medial thickness and smooth muscle cell number, and no intimal proliferation or adventitial inflammation. Thus, heparin-like molecules appear to act in concert with low doses of cyclosporin in preventing rejection-induced arterial wall remodeling in an experimental model of aortic allograft in rats, and their effects are independent of blood pressure.

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KEY WORDS • heparin • cyclosporin • rats • graft rejection

Chronic arterial rejection is the major complication that arises from clinical organ transplants. In particular, posttransplant accelerated coronary arteriosclerosis is the main chronic complication after cardiac transplantation.1 Cyclosporin treatment has reduced the short-term rejection mortality rate, but posttransplant accelerated coronary arteriosclerosis appears to be the predominant pathology that limits the survival of long-term heart transplant patients,2 and this seems to be only slightly influenced by cyclosporin treatment.Billingham2 found that almost 40% of patients were suffering from this disease 3 years after transplantation. It is the sole reason for retransplantation after 1 year, and the third largest cause of mortality in cardiac transplantation. Bieber et al13 showed that posttransplant accelerated coronary arteriosclerosis involves the three layers of the arterial wall, with fibrointimal proliferation, medial necrosis, and adventitial inflammatory cell infiltration. The histopathological lesions of chronic posttransplant accelerated arteriosclerosis also occur after most other types of organ transplants, particularly those of the kidney.4

Posttransplant arteriosclerosis develops in response to immune injury of the arterial wall. This immunological injury to and response of the arterial wall can be experimentally reproduced by the aorto-aortic allograft model in rats.5-8 This experimental model is more
useful than experimental heart transplantation, as the
former focuses on the pathological structure of the
arterial wall. The longitudinal and regular morphology
of the abdominal aorta is also suitable for quantitative
analysis of arterial wall injury and response, whereas
the irregular and complex anatomy of the coronary
circulation is not. In this aortic allograft model, adven-
titial inflammatory cell infiltration provides evidence of
the rejection process, injury is indicated by medial
smooth muscle cell necrosis and breaks in the medial
extracellular matrix, and intimal cellular proliferation
and extracellular matrix accumulation are a measure of
the vascular wall response. Hypertension increases the
intimal proliferative response, and vasodilator treat-
ment, which induces a fall in blood pressure, decreases
the intimal thickness in this model in the same way that
hypertension and its treatment modify the intimal re-
response to balloon-induced deendothelialization and
smooth muscle stretch. High doses of cyclosporin prevent immune injury in
this model of the aortic allograft in rats. Schmitz-Rixen
et al not only reported that cyclosporin was beneficial in
preventing medial injury in aortic allografts in rats but
also showed that it had no major effect on intimal
proliferation. These data have been recently confirmed
by the study of Mennander and coworkers that de-
scribes the absence of any beneficial effect of cyclosporin
on intimal proliferation in arterial allograft rejection.
These experimental data fit well with the clinical data
reporting the absence of any beneficial effect of cyclo-
sporin treatment on accelerated posttransplant arterio-
sclerosis. Long-term treatment with cyclosporin is also
frequently associated with hypertension.

Heparin and low-molecular-weight, heparin-like mol-
ecules have been reported to inhibit the intimal prolif-
ERATIVE response to mechanical arterial wall injury in vivo. Lider et al have also recently reported that a low-
molecular-weight, heparin-like molecule can partially
prevent skin allograft rejection in mice. Experimental
posttransplant arteriosclerosis appears to be a suitable
target for heparin and heparin-like molecules because
heparin can act on the two components of arterial graft
rejection: immune injury and the intimal response. We
have, therefore, examined the possibility that a low-
molecular-weight, heparin-like molecule can act together
with a low dose of cyclosporin to prevent chronic arterial
graft rejection. This hypothesis was tested in an exper-
imental design with our model of abdominal aortic al-
lograft rejection in normotensive (Wistar-Kyoto [WKY])
and hypertensive (spontaneously hypertensive rat [SHR])
histoincompatible inbred rats.

Methods

Experimental Design

A total of 100 male SHRs (body weight, 190±30 g)
and 100 male normotensive WKY control rats (body
weight, 200±30 g), all 8 weeks old, were purchased from
Iffa-Credo, Lyon, France. All animal care and use
procedures were in accordance with the European
Community Standards on the Care and Use of Labora-
tory Animals (No. 86/609/EEC). Aortas were transplanted by
using microsurgical techniques. Rats were anesthetized
with pentobarbital (5 mg/100 g body wt i.p.). Two
animals underwent operation simultaneously, one as
the donor of the aortic graft and the other as the recipient.
While it was viewed under the operating microscope, the
infrarenal aorta was exposed from the left renal vein to the aortic bifurcation via a midline
laparotomy. Any aortic branches in this segment were
identified and ligated. Two microclips were placed, one
below the renal arteries and the second above the aortic
bifurcation. A 1-cm segment of aorta was removed from
the donor animal and washed with saline. A similar
resection of the infrarenal aorta was performed in the
recipient rat. The donor segment was immediately
inserted into the aorta of the recipient with end-to-end
interrupted anastomoses using 9/0 nonabsorbable
monofilament nylon sutures. Cold ischemic time was 30
minutes. No preservation solution other than saline was
used. The patency of each graft was evaluated. No acute
secondary graft thrombosis occurred during any of the
experiments. The laparotomy was closed, and the rat
was returned to its cage. All rats were fed a standard
diet, and water was provided ad libitum.

In our first study, we demonstrated that the isografts
did not significantly differ from the aortas of sham-
operated control animals. Therefore, 10 syngeneic
isografts were performed for each strain (SHR and
WKY), which served as controls. Forty SHRs were
allografted with aortas from WKYs, and 40 WKYs
were treated similarly with aortas from SHRs. Ten animals of
each recipient strain were treated with cyclosporin, 10
were treated with heparin, 10 were treated with heparin
plus cyclosporin, and 10 allografted, vehicle-treated rats
served as the allografted control group. This experi-
mental protocol was designed to use a two-way analysis of
variance (ANOVA) to test the effect of each drug alone
and of their combination on the different parameters. In
a two-factor ANOVA, a statistically significant positive
interaction reflects a synergistic effect of the two treat-
ments, whereas the absence of a significant interaction
reflects only an additive effect of the two treatments. All
treatments were begun 1 week before surgery and
continued daily for 8 weeks. Cyclosporin A (Sandoz,
Basel, Switzerland), dissolved in oil, was given by sub-
cutaneous injection (2 mg/kg body wt daily). The low-
molecular-weight, heparin-like molecule was given by
continuous infusion (Lab Choya France IC 86 1772)
with an osmotic infusion pump (Alzet, Alza Corp., Palo
Alto, Calif.) that was placed in the subcutaneous tissue
in the back. Active pumping began approximately 4
hours after implantation. The pumps contained 2 ml of
solution that was pumped at a rate of 5 μl/hr. Heparin
was infused for 8 weeks. Infusion pumps were
replaced every 2 weeks while the rats were under ether
anesthesia. Vehicle-treated animals received only sub-
cutaneous injections of oil. Blood cyclosporin concen-
tration was measured on total hemolysed blood samples
by a fluorescence polarization immunoassay (Abbott
TDX analyzer) using a monoclonal antibody.

Measurement of Aortic Graft Volumes

Eight weeks after graft microsurgery, the rats were
anesthetized with inactin (0.1 mg/100 g body wt i.p.),
and the aortic graft volumes were determined by in vivo
catheterization and isolation of the grafted segments.
The catheter was filled with Evans blue dye and con-
ected to a pressure transducer for simultaneous mea-
surement of pressure and volume. The volume of the
aortic segment at a pressure of 75 mm Hg was recorded for each rat.

Morphological Studies
At the end of the pressure measurements the 1 cm of
grafted abdominal aorta was fixed, in situ under anes-
thesia by infusion of 10% buffered formalin solution at
mean arterial pressure. The graft was removed, dehy-
derated, and embedded in paraffin for light microscopy.
Three successive sagittal sections (5 μm) were cut and
stained, one for each of the structures in the aortic wall
that was studied. Collagen fibers were stained with
Sirius red and elastin/orcein, and the nuclei were
stained with hematoxylin after periodic acid oxidation.
Slides were analyzed in an automatic image processor
(NS 1500, Nachet-Vision, Paris, France). An addi-
tional section was stained with Masson’s trichrome for
standard histological examination. Algorithms were de-
veloped to analyze the structures that were stained on
each of the three successive sections. The first algorithm
analyzed the mean medial thickness by measuring the
distance between the internal and external elastic lam-
inae (20 measurements on each section). The medial
elastin network was analyzed in terms of the number of
elastic laminae by measuring 10 fields in each section.
The second algorithm analyzed the collagen matrix by
measuring the relative area, density, and mean thick-
ness of collagen fibers in 20 contiguous fields in each
Sirius red-stained section. The third algorithm counted
the number of nuclei within 20 fields in each section and
measured the mean area of each nucleus. Repetitive
measurements were performed, pooled, and averaged
each animal. Similar measurements of nuclear den-
sity and collagen were performed for determination of
intimal proliferation.

Statistical Analysis
Results are expressed as mean±SD. The experimental
design allowed a factorial two-way ANOVA to be used
that would show the effects of rat strain and treatment and
would compare the effects of the treatments. Linear
regression curves and correlation coefficients were ob-
tained by the least-squares method. Analysis of covari-
ance was performed to test the influence of the various treat-
ments on the relation between the density of adventitial
inflammatory cells and the density of medial smooth
muscle cells. In a pilot study, a nested ANOVA was
performed to determine the intraindividual variance of
the histomorphometric parameters in aortas from WKYs
and SHRs that were given the two types of treatment for
3 months. Data were analyzed according to different levels
of hierarchy: two strains, two treatments, 12 rats from each
strain, and 20 fields from each section that were subjected
to image analysis. The total number of measurements for
each parameter was 960 (2x2x12x20). The results
showed that for quantification of thickness, elastin, colla-
gen, and nuclei, the observed variance was due to differ-
ences in strains and/or treatment but not to differences
between rats from the same group or between fields from
the same section.

Results

Blood Pressure and Cyclosporin Concentration
All SHR recipients had significantly higher blood
pressures than did the WKYs. Allografts did not change
blood pressure in SHRs or WKYs. The treatments did
not alter the blood pressure in the two strains (Table 1).
A small “blank” of cyclosporin concentration was
detected in the blood of control allograft and heparin-
treated animals (Table 1). Cyclosporin alone increased
the blood level of cyclosporin, whereas the combination
of cyclosporin with heparin gave measurements that
were higher than control values but lower than those
with cyclosporin alone.

Aortic Volumes at 75 mm Hg
Allografted rats of both strains had a significantly
increased aortic volume (F=44, p<0.001) than did
isografted rats. There were also differences between the
two recipient groups. The grafted aortic volumes in
SHR recipients were greater than those in WKY recip-
ients (F=10, p<0.001).

Cyclosporin or heparin alone did not change the
allografted aortic volumes (F=0.6 for each treatment).
In contrast, the allografted aortic volumes of rats
treated with cyclosporin plus heparin were lower than
those of untreated rats (F=6.2, p=0.01), rats given
cyclosporin alone (F=4.5, p<0.05), or rats given heparin
alone (F=5.4, p<0.05) and did not differ from those of
isografted rats (F=2.7, NS). The cyclosporin and hepa-
rin interacted to statistically lower the volumes of
allografted aortas (F=4.2, p<0.05) (Figure 1).

Morphological and Morphometric Results
Effect of the allograft. A monolayer of endothelial cells
was present at the luminal pole of each arterial graft in
all experimental groups. All untreated aortic allografts
showed evidence of rejection under the light microscope
(Masson’s trichrome staining). The histological signs of
arterial graft rejection were adventitial cellular infiltrat-
ton, intimal thickening, and medial necrosis 2 months
after transplantation. The lesions seen in this study were

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Blood Pressure (mm Hg)</th>
<th>Cyclosporin (ng/ml)</th>
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</thead>
<tbody>
<tr>
<td>WKY (n=40)</td>
<td>Control (n=20)</td>
<td>84±3</td>
<td>6.4±2.1</td>
</tr>
<tr>
<td></td>
<td>Cyclosporin (n=20)</td>
<td>95±7</td>
<td>309±10</td>
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<tr>
<td></td>
<td>Heparin (n=20)</td>
<td>82±7</td>
<td>5.2±0.8</td>
</tr>
<tr>
<td></td>
<td>Cyclosporin plus heparin (n=20)</td>
<td>95±8</td>
<td>36.8±4.4</td>
</tr>
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</table>

Values are mean±SD. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats. ANOVA, analysis of variance.
similar to those of other studies. Adventitial cellular infiltration indicated rejection, medial necrosis reflected the injury due to rejection, and intimal thickening reflected the arterial wall response (Figure 2).

The qualitative results were confirmed by the quantitative data. Medial thickness was lower in allografts than in isografts as a result of the disappearance of medial smooth muscle cells and of the decrease in collagen density and elastic laminae (Table 2). The intimal thickness of allografts was greater than that of the isografts (Table 3). There were also some differences between the two strains; these have been described in more detail in our first study. Allografted aortas were characterized by a significant increase in adventitial cell density (Table 4). The relation between rejection and arterial wall injury and response was indicated by the positive correlation between the intima/media thickness ratio and the density of adventitial inflammatory cells in untreated allografts ($r=0.53$, $F=5.5$, $p<0.01$) and the positive correlation between adventitial inflammatory cell and medial smooth muscle cell densities ($r=0.7$, $F=12.5$, $p<0.005$).

Effect of cyclosporin alone. Long-term treatment with cyclosporin significantly reduced medial allograft injury (Table 2). Medial thickness was significantly greater in cyclosporin-treated allografts than in untreated allografts but remained lower than in isografts ($F=6.6$, $p<0.01$). The number of remaining living cells in the media and medial collagen density were also higher. Last, the number of elastic laminae increased to a value similar to that of isografts. The intimal proliferation thickness of cyclosporin-treated rats was similar to that of untreated allografts (Table 3). The number of myointimal cells was significantly increased, but there was no significant change in collagen density from that of untreated allografts. Low doses of cyclosporin alone significantly
increased the number of adventitial inflammatory cells 2 months after the induction of the model (Table 4). In untreated allografts, there was a positive correlation between intima/media thickness ratio and the density of inflammatory adventitial cells \( (r=0.5, F=5.3, p<0.05) \). In this group, there was also a positive correlation \( (r=0.46, F=4.6, p<0.05) \) between medial smooth muscle cell and adventitial inflammatory cell densities.

**Effect of a heparin-like molecule.** Long-term treatment with a heparin-like molecule also significantly reduced medial injury (Table 2). Medial thickness was significantly higher in heparin-treated allografts than in untreated allografts but was not different from that in the isografts \( (F=3.3, \text{NS}) \). The number of living cells remaining in the media, collagen density, and the number of elastic laminae were also increased. The effects of heparin treatment on medial protection against allograft injury were similar to those of low doses of cyclosporin alone. Heparin treatment greatly decreased intimal proliferative thickness compared with the untreated allograft (Table 3). Collagen density significantly decreased, but myointimal cell density increased. As with low doses of cyclosporin, the number of adventitial inflammatory cells was significantly higher in allografts that were treated with heparin alone than in untreated allografts (Table 4). There was no correlation between intima/media thickness ratio and the density of inflammatory adventitial cells \( (F=1, \text{NS}) \). As in the untreated and cyclosporin-treated allografts, there was a positive correlation \( (r=0.5, F=4.5, p<0.05) \) between medial smooth muscle cell and adventitial inflammatory cell densities.

**Effect of heparin-like molecule plus cyclosporin.** The effect of the combined treatment on injury and response in the allografted arterial wall was spectacular. Medial thickness was greater \( (F=104, p<0.001) \) than in the untreated allograft. The number of remaining living cells \( (F=381, p<0.001) \) and elastic laminae \( (F=88, p<0.001) \) and collagen density \( (F=17, p<0.001) \) in the media were also increased.

Except for medial thickness \( (F=0.40, \text{NS}) \), the effect of the combination of both treatments on medial smooth muscle cell \( (F=40, p<0.001) \), collagen \( (F=5.5, p<0.05) \), and adventitial inflammatory cell density was no different from that in the isografts.
p<0.01), than that of isografted aortas.

The medial necrosis is the consequence of the immune wall response. The correlation between adventitial inflammation by inflammatory cells and the ratio of intimal proliferation to medial thickness in untreated allografts demonstrates one of the major features of the model, the importance of inflammation in the injury and response to chronic vascular immune aggression. Nevertheless, Mennander et al. reported that adventitial inflammation was predominant during the first month after arterial grafting in this model, a time when medial smooth muscle cells were still present. After this time, the adventitial inflammation decreased in parallel with the disappearance of medial smooth muscle cells. Our data confirm this result by showing a positive correlation between the number of medial smooth muscle cells and the adventitial inflammatory cell density in untreated allografts. These data show that not only endothelial cells but also medial smooth muscle cells participate in the chronic vascular rejection process.

The combination also interacted to prevent adventitial inflammation (Table 4). In contrast with cyclosporin and heparin alone, the density of inflammatory adventitial cells was significantly lower in allografts that were treated with cyclosporin plus heparin (F=180, p<0.001). Nevertheless, there was no statistical interaction between heparin and cyclosporin in terms of a decrease in intimal thickness (Table 3). Therefore, the effect of cyclosporin plus heparin in preventing intimal thickening appears to be more of an additive than a synergistic effect. Cyclosporin and heparin interacted to reduce the number of smooth muscle cells and collagen density of the intimal proliferation.

The combination also interacted to prevent adventitial inflammation (Table 4). In contrast with cyclosporin and heparin alone, the density of inflammatory adventitial cells was significantly lower in allografts that were treated with cyclosporin plus heparin (F=21, p<0.001) than in untreated allografts. There was a tendency toward a negative correlation ($r=-0.41, F=3.7, p=0.07$) between medial smooth muscle cell and adventitial inflammatory cell densities in this group. Analysis of covariance showed the absence of any significant difference between isografts and the heparin plus cyclosporin–treated allografts ($F=0.1, NS$). Therefore, there was a negative correlation between adventitial and medial cell densities in isografts and heparin plus cyclosporin–treated allografts (Figure 3). In contrast, there was a general positive correlation between adventitial and medial cell densities in untreated, cyclosporin–treated, and heparin–treated allografts. Analysis of covariance showed that the heparin plus cyclosporin treatment had a significant influence on the relation between adventitial inflammatory cell and smooth muscle cell densities compared with cyclosporin or heparin alone.

**Discussion**

Experimental chronic arterial allograft rejection involves three types of lesions: medial necrosis, intimal proliferation, and inflammatory cell infiltration. The present study confirms our previous histological descriptions and those of Schmitz-Rixen et al., Mennander et al., and Häyry et al. Chronic arterial allograft rejection is a model of immune response–induced arterial wall injury. The medial necrosis is the consequence of the immune injury, whereas intimal proliferation represents the arterial wall response. The correlation between adventitial infiltration by inflammatory cells and the ratio of intimal proliferation to medial thickness in untreated allografts demonstrates one of the major features of the model, the importance of inflammation in the injury and response to chronic vascular immune aggression. Nevertheless, Mennander et al. reported that adventitial inflammation was predominant during the first month after arterial grafting in this model, a time when medial smooth muscle cells were still present. After this time, the adventitial inflammation decreased in parallel with the disappearance of medial smooth muscle cells. Our data confirm this result by showing a positive correlation between the number of medial smooth muscle cells and the adventitial inflammatory cell density in untreated allografts. These data show that not only endothelial cells but also medial smooth muscle cells participate in the chronic vascular rejection process.

**Table 4. Effects of Rat Strain, Graft Type, and Treatment on Adventitial Cellular Infiltration 2 Months After Grafting**

<table>
<thead>
<tr>
<th></th>
<th>Isograft (n=20)</th>
<th>Control (n=20)</th>
<th>Cyclosporin (n=20)</th>
<th>Heparin (n=20)</th>
<th>Cyclosporin plus heparin (n=20)</th>
<th>Allograft versus isograft</th>
<th>Cyclosporin effect</th>
<th>Heparin effect</th>
<th>Interaction of cyclosporin x heparin</th>
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<tr>
<td>Adventitial cell density (No./field)</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>WKY (n=50)</td>
<td>15±5</td>
<td>26.5±4</td>
<td>56±11</td>
<td>56±12</td>
<td>12.2±8</td>
<td>F=37</td>
<td>F=25</td>
<td>F=15</td>
<td>F=72</td>
</tr>
<tr>
<td>SHR (n=50)</td>
<td>16±2</td>
<td>58.4±8</td>
<td>91.2±7</td>
<td>71.7±10</td>
<td>22.4±7</td>
<td>p&lt;10^-3</td>
<td>p&lt;10^-3</td>
<td>p&lt;10^-3</td>
<td>p&lt;10^-3</td>
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</table>

Values are means±SD. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; ANOVA, analysis of variance.
Cyclosporin Effects

Low doses of cyclosporin protect the aortic media against destructive processes. An increase in medial smooth muscle cell density and partial protection of the extracellular matrix indicate the beneficial effect of cyclosporin in preventing arterial wall immune injury. These results are similar to those of Schmitz-Rixen et al.\(^5\) and Mennander et al.\(^2\) who used different doses of cyclosporin. Paradoxically, in our study low doses of cyclosporin apparently increased adventitial inflammation. This result is probably due to the chronology of the rejection process. The evidence of rejection probably decreases in untreated allografts in relation to the disappearance of smooth muscle cellular antigens 2 months after grafting.\(^7\) In contrast, low doses of cyclosporin could delay the appearance of rejection, so that the inflammation appeared greater in cyclosporin-treated animals than in untreated allografts 2 months after grafting. This inflammation could be related to the persistence of medial cellular antigens, as suggested by the positive correlation between medial smooth muscle cell and adventitial inflammatory cell densities. Nevertheless, the fibrointimal proliferative response was still present. This phenomenon may be analogous to the cyclosporin-resistant chronic obstructive arteriosclerosis that is seen in organ transplantation, particularly in heart transplantation.\(^3\)

Jonasson et al.\(^20\) have reported a decrease in intimal proliferative thickness in carotid artery deendothelialization in rats with high doses of cyclosporin. In contrast, Ferns et al.\(^21\) did not find any change in intimal proliferation thickness after cyclosporin treatment in a carotid artery deendothelialization model in cholesterol-fed rabbits. In vitro, cyclosporin decreases the smooth muscle cell proliferation rate in a dose-dependent fashion.\(^22\) Therefore, clinical and experimental data tend to show that cyclosporin alone does not prevent the arteriosclerotic lesions of the arterial wall rejection process. Our data for low doses of cyclosporin confirm this general agreement.

Heparin Effects

Low-molecular-weight, nonanticoagulant, heparin-like molecules had two beneficial effects: one on the vascular wall injury and the other on the intimal proliferative response. Nevertheless, as with low doses of cyclosporin, heparin alone increased the density of adventitial cellular inflammation 2 months after grafting. The reason is probably similar to that suggested for cyclosporin: a delayed peak of rejection due to the persistence of medial cellular antigens.

Medial protection resulted in persistent medial smooth muscle cells and less destruction of the extracellular matrix. The effects of low-molecular-weight, heparin-like molecules on the rejection process were first described by Lider et al.\(^15\) in a mouse skin-allograft model. They reported that low doses of heparin-like molecules without anticoagulant effects altered T-lymphocyte functions in vitro\(^22\) and in vivo.\(^15\) Reports indicate that T lymphocytes have an endoglycosidase that specifically degrades the heparan sulfate side chains of the extracellular proteoglycans in vitro.\(^23\)-\(^25\) Heparanase activity can generally be blocked in vitro by heparin.\(^24\),\(^26\),\(^27\) Inhibition of endogenous heparanase by a suitable dose of heparin limits tissue lymphocyte traffic. Others have shown anti-inflammatory effects of heparin\(^28\),\(^29\) or heparin-like molecules\(^30\) in several models. Bradfield and Born\(^31\) described the inhibition of lymphocyte emigration from blood into lymphoid tissue by heparin-like molecules in vivo. Heparin also modulates the complement system.\(^32\) Jaques\(^33\) reported a C1q-C1r junction inhibitory effect and a C1 esterase stimulatory effect in vitro.\(^33\),\(^34\) Other anticomplement effects have been attributed to heparin.\(^36\),\(^37\) Last, heparin-like molecules increase the negative charge on endothelial cells and lymphocytes, thus impeding lymphocyte migration.\(^38\) One or more of these actions could explain the partial protection from immune injury by low-molecular-weight, heparin-like molecules.

Heparin may also act on the two components of intimal proliferation, the smooth muscle cells and the extracellular matrix. Intimal thickness is clearly decreased by heparin. Clowes and Karnowsky\(^44\) reported that heparin prevented intimal proliferation, and more recently Snow et al.\(^37\) obtained similar results in a model of carotid artery deendothelialization in rats. Nonanticoagulant, low-molecular-weight, heparin-like molecules were as effective as anticoagulant molecules in preventing intimal proliferation.\(^38\) In vitro, heparin inhibits smooth muscle cell migration and proliferation.\(^39\) Our present data also indicate that heparin-like molecules can limit intimal proliferation in response to arterial wall immune injury. The content of intimal proliferation was also modified by heparin. Collagen density decreased and cell density increased, indicating that heparin acts more on cell tropichity than on the cell proliferative capacity. These results on intimal proliferation are in agreement with those of Snow et al.\(^37\) who reported a decrease in collagen density with heparin in a model of carotid artery deendothelialization in rats. In vitro, Tan et al.\(^40\) described a decrease in collagen synthesis by smooth muscle cells in culture after the addition of heparin.

Castellot et al.\(^41\) reported that the production of a heparin-like molecule by bovine aortic endothelial cells in culture could inhibit smooth muscle cell proliferation in vitro. Heparan sulfates could be synthesized by normal quiescent endothelial cells and so prevent smooth muscle cell migration and proliferation. Endothelial injury may disrupt this physiological balance.\(^42\)

Heparin Plus Cyclosporin

The heparin plus cyclosporin combination dramatically protects the allografted arterial wall against rejection. Medial injury is prevented, and there is no intimal proliferation. The medial thickness is preserved, as are the elastin and collagen contents. Medial smooth muscle cell density remains similar to that of the isografted aortic wall. Intimal proliferation disappears. Despite the persistence of large amounts of medial cellular antigens, adventitial inflammatory infiltration is also lower than in untreated and cyclosporin- or heparin-treated allografts (covariance analysis).

Chronic arterial rejection is a major determining factor in the outcome of organ transplants. The immune-response remodeling of the arterial wall involves both medial injury and intimal proliferation. Cyclosporin alone, by its specific effect on T-helper lymphocytes, lowers immunological rejection and medial injury but has no major beneficial effect on intimal proliferative
The beneficial effect of heparin plus cyclosporin cannot be attributed to an increase in cyclosporin concentration because blood cyclosporin levels decreased in the presence of heparin. The positive statistical interaction between the effects of cyclosporin and heparin on two components of graft rejection, adventitial cellularity and persistence of medial smooth muscle cells, suggests that the two treatments act synergistically on rejection injury. In contrast, cyclosporin and heparin do not interact in the prevention of intimal thickening. Therefore, the beneficial effect of the combination on the intima predominantly involves an additive effect rather than a synergistic effect.

References

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