Effect of Combined Locally Delivered Growth Factors and Systemic Sildenafil Citrate on Microrecanalization in Biodegradable Conduit for Vas Deferens Reconstruction

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OBJECTIVE
To investigate the effect of the combination of locally delivered growth factors and oral sildenafil citrate on cross-conduit microrecanalization.

METHODS
A total of 42 rats were divided into 7 groups. Of the 42 rats, 6 underwent bilateral vasectomy and bilateral end-to-end vasovasostomy and 12 underwent bilateral vasectomy. Of the latter 12, 6 received sildenafil citrate orally (10 mg/kg/d) for 24 weeks and 6 received placebo. A total of 24 rats underwent bilateral vasectomy and bilateral reconstruction with implantation of a 5-mm biodegradable conduit that bridged the 2 vasal ends. Of the 24 rats with conduits, 12 also had 250 pg of transforming growth factor-β and 12.5 pg of platelet-derived growth factor-β sustained release nanoparticles placed in immediate proximity to the conduit. The remaining 12 rats with conduits (6 without growth factors and 6 with growth factors) also received sildenafil citrate orally (10 mg/kg/d) for 24 weeks; the others received placebo. The reconstructed segments were harvested for histologic examination at 24 weeks.

RESULTS
Five of 6 primary vasovasostomy and no vasectomy-only rats sired litters. Significantly more microcanals per conduit were observed in rats receiving sildenafil citrate: without growth factors, 3.9 vs. 0 canals/conduit ($P < 0.001$); with growth factors, 5.5 vs. 0.25 canals/conduit ($P < 0.001$). The rats receiving sildenafil citrate with growth factors showed a trend toward more microcanals per conduit than the rats receiving sildenafil citrate without growth factors (5.5 vs 3.9; $P = .10$). Rats receiving growth factors but no sildenafil citrate did not produce more canals than the rats receiving neither growth factor nor sildenafil citrate (0.25 vs 0; $P = NS$).

CONCLUSION
Orally administered sildenafil citrate enhances formation of microcanalization after postvasectomy reconstruction using a biodegradable conduit in a rat model. Locally delivered growth factors appear to increase the number of microcanals.

In the United States alone, >500 000 men choose vasectomy to address undesired fertility every year. In this group, ≤6% eventually request a reversal. Additionally, iatrogenic vas deferens injury can require surgical correction to prevent loss of fertility. In some situations, long segments of obstructed vas deferens, because of previous vasectomy, iatrogenic injury, or congenital conditions, prevent primary vasovasostomy. To bridge the obstructed vas deferens, our group has developed a biodegradable conduit to guide the growth of spontaneously appearing microcanals, with the goal of restoring vas deferens continuity. We have previously shown that certain growth factors, specifically transforming growth factor (TGF)-β and platelet-derived growth factor (PDGF)-β, are elevated at the site of vasectomy and might be related to microcanal growth. Our group has also shown that oral sildenafil citrate, a phosphodiesterase inhibitor that increases vascular endothelial growth factor expression and angiogenesis in a rat model of coronary ischemia, increases the number of microcanals present in the biodegradable conduits.

In this project, we hypothesized that oral administration of sildenafil citrate combined with locally de-
lerived growth factors would lead to enhanced micro-
canal formation in implanted biodegradable vas deferens conduits.

**MATERIAL AND METHODS**

**Conduits**
The biodegradable conduits were designed and constructed at
Iowa State University (Ames, IA), as previously described (Fig.
1).6 In brief, porous conduits were constructed by dissolving
poly-d,l-lactide (PDLA; Lactel, Birmingham Polymers, Pel-
ham, AL) in chloroform and then adding sieved sodium chloride to obtain the finished conduits with 75% porosity. Polyvinyl alcohol-coated glass capillary tubes were dipped into the PDLA/salt suspension, and the tubes were soaked in water to remove the sodium chloride and polyvinyl alcohol. The conduits were dried in a desiccator to complete the process.

**Cytokine-Containing Nanoparticles**
The nanoparticles were designed and constructed at the
University of Iowa (Iowa City, IA). Two milligrams of poly-lacti-
coc-glycolic acid was dissolved in 50 μL dichloromethane; 5 μL
of TGF-β solution equivalent to 500 ng of the growth factor (or 5 μL of PDGF-β) in phosphate-buffered saline was mixed with 5 μL of 1% polyvinyl alcohol. The aqueous peptidase solution was emulsified into the organic polymer solution using an ultrasonicator probe (10 W power) for 10 seconds. The primary emulsion was rapidly emulsified into 500 μL of 1% polyvinyl alcohol solution using an ultrasonicator probe (10 W power) for 10 seconds. This emulsion was processed using a magnetic stirrer until complete evaporation of dichloromethane. The particles were collected by centrifugation at 14,000 rpm for 10 minutes. The particles were then suspended in 300 μL phosphate-buffered saline. We chose a concentration of 251 pg/conduit for TGF-β and 12.5 pg/conduit for the PDGF-β particles according to our previous data.9

**Rats and Vasectomy Surgery**
The Institutional Animal Care and Use Committee of the
University of Iowa approved the study protocol. A total of 42
male Sprague-Dawley rats were used in the experiment and
were divided into 7 groups of 6 rats (Table 1). Of the 42 rats, 6
underwent bilateral vasectomy followed by bilateral end-to-end
vasovasostomy with 10-0 nylon suture, 12 underwent only
bilateral vasectomy, and 24 underwent bilateral vasectomy fol-
lowed by bilateral reconstruction with implantation of 5-mm
biodegradable conduits that bridged the vasal ends (48 total
conduits). The conduits were sutured in place using 10-0 nylon
stitches. Of the 24 rats with conduits implanted, 12 (24 con-
duits) also had 250 pg TGF-β and 12.5 pg PDGF-β in sus-
tained-release nanoparticles placed in immediate proximity to
the conduit. Also, 6 rats with implanted conduits and no
growth factors and 6 rats with implanted conduits plus growth
factors were then given oral sildenafil citrate (10 mg/kg/d) for
24 weeks. The others were given placebo. This dose of sildenafil
was determined from a study by Ding et al,7 showing that rats
reared with that dose had increased angiogenesis. Also, 6 of
the rats who had undergone vasectomy only were given oral sild-
enafl citrate (10 mg/kg/d) for 24 weeks and 6 of the rats who had
undergone vasectomy only were given placebo. All rats were
housed with a female rat for 2 estrous cycles (10 days) starting
2 weeks after surgery. After 24 weeks, all the rats were killed,
and the reconstructed vasal segments were harvested and serially
sectioned for histologic examination. At this surgery, a
sample of vasal fluid from the abdominal end of the vas was
obtained and mixed with 1 drop of phosphate-buffered saline
for immediate identification of motile sperm by light micros-
opy. The harvested reconstructed segments were serially sec-
tioned at 1-mm intervals and stained with hematoxylin-eosin
for microscopic analysis by a single pathologist who was un-
aware of the treatment groups.

**Statistical Analysis**
All statistical analyses was conducted using StatView, version
5.0.1 (SAS Institute, Cary, NC). Student’s t test was used to
compare the mean values, and a z test was used to compare the
percentages. The variables were assumed to be normally distrib-
uted. P < .05 was considered significant, and all P values were
2-sided.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Intervention</th>
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<tbody>
<tr>
<td>1 (n = 6)</td>
<td>Vasectomy with immediate vasovasostomy</td>
</tr>
<tr>
<td>2 (n = 6)</td>
<td>Vasectomy, oral sildenafil citrate</td>
</tr>
<tr>
<td>3 (n = 6)</td>
<td>Vasectomy, oral placebo</td>
</tr>
<tr>
<td>4 (n = 6)</td>
<td>Vasectomy with immediate conduit implantation, growth factors, no sildenafil citrate</td>
</tr>
<tr>
<td>5 (n = 6)</td>
<td>Vasectomy with immediate conduit implantation, growth factors, plus sildenafil citrate</td>
</tr>
<tr>
<td>6 (n = 6)</td>
<td>Vasectomy with immediate conduit implantation, no growth factors, no sildenafil citrate</td>
</tr>
<tr>
<td>7 (n = 6)</td>
<td>Vasectomy with immediate conduit implantation, no growth factors, plus sildenafil citrate</td>
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**Table 1. Experimental groups and interventions**

Figure 1. Scanning electron microscope image of polymer conduit. Bar = 200 μm.
RESULTS

Live Births and Semen Analysis
No pregnancies were sired by any rat with implanted PDLA conduits. Of the 6 rats in the primary vasovasostomy group, 5 (83%) sired litters, and no rat receiving only a vasectomy sired a litter. No motile sperm was observed in the distal vasal fluid of the rats in the grafted groups. Motile sperm was present in all the rats in the primary vasovasostomy group.

Gross and Histologic Analysis
At 24 weeks, all PDLA grafts were easily identifiable, grossly intact, and sealed to the vas at both ends. Grossly, the rats that had received oral sildenafil citrate had more vasculature surrounding the conduits than the rats who had not received oral sildenafil citrate (Fig. 2A). Histologic examination of the PDLA conduits revealed the presence of a granulomatous inflammatory response within the lumens. Also identified were multiple microcanals lined with a distinct layer of cuboidal epithelial cells within the lumens of the grafts, such as has been seen previously (Fig. 2B).

Table 2. Average number of microcanals per conduit

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Average Canals/Unit (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth factors, no sildenafil citrate</td>
<td>0.25</td>
</tr>
<tr>
<td>Growth factors, plus sildenafil citrate</td>
<td>5.5</td>
</tr>
<tr>
<td>No growth factors, no sildenafil citrate</td>
<td>0</td>
</tr>
<tr>
<td>No growth factors, plus sildenafil citrate</td>
<td>3.9</td>
</tr>
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Microcanals
The average number of microcanals per conduit is listed in Table 2. Of the 24 conduits harvested from the rats not given growth factors, significantly more microcanals per conduit were observed in the rats receiving sildenafil citrate (3.9 vs 0 canals/conduit; P < .001). Of the 24 conduits from rats receiving growth factors, significantly more canals were also observed in the sildenafil citrate group (5.5 vs 0.25 canals/conduit; P < .001). Those rats receiving growth factor but no sildenafil citrate did not produce more canals compared with the rats receiving neither growth factor nor sildenafil citrate (0.25 vs 0; P = NS). Although rats receiving sildenafil citrate and growth factors did produce more canals than rats receiving sildenafil citrate alone, the difference was not statistically significant (5.5 vs 3.9, P = .10). No complete cross-conduit canals were present in any group.

COMMENT
Currently, few surgical options exist to address long segments of obstructed vas deferens when a primary vasovasostomy cannot be completed. In these cases, when the vas deferens cannot be successfully reconstructed, patients are forced to consider surgical sperm retrieval and assisted reproductive techniques to achieve pregnancy. As an alternative, our group has developed a biodegradable PDLA conduit to be used in vas deferens reconstruction. We first showed that microcanals could be readily identified in the conduits 12 weeks after simple implantation.6 We then found that concomitant use of oral sildenafil citrate increased the number of microcanals present in the conduits after 16 weeks, but none of the microcanals spanned the entire 5-mm conduit.4 We also showed that TGF-β and PDGF-β were elevated at the site of vasectomy,3 leading us to hypothesize that these growth factors might be important in microcanal development. TGF-β and PDGF-β stimulate proliferation of fibroblasts and smooth muscle cells, as well as having a chemotactic role.8 Additionally, TGF-β might have a role in sperm maturation.9 Because of these collective observations, we hypothesized that the combination of oral sildenafil citrate and local delivery of TGF-β and PDGF-β would further increase the degree of recanalization present in the conduits.

Because growth factors have very short half-lives when exposed to serum, we used a nanoparticle delivery system to achieve continuous local release of TGF-β and PDGF-β. Nanoparticle delivery systems sequester biologically active molecules, which are then released over time as the nanoparticle superstructure dissolves.10

Figure 2. (A) Gross operating microscope images of conduits in situ from rats that received sildenafil citrate. (B) Photomicrograph cross section of conduit from rat that received both growth factors and sildenafil citrate.
Our work has shown that concomitant use of oral sildenafil citrate and locally delivered growth factors does increase microcanal formation; however, even after 24 weeks, no microcanals had completely crossed the conduits. There are several potential explanations for this. One is that 24 weeks was not a sufficient amount of time to complete this process. Related to this is that we only gave growth factor-laden nanoparticles once. Thus, it might be necessary to administer the nanoparticles a second time to ensure sufficient growth factor is present to facilitate complete canal formation. Alternatively, it would be interesting to examine the rate of recanalization in conduits directly impregnated with growth factors. Finally, we noted a high degree of inflammation within the conduits, which might have counteracted the effect of the growth factors. Decreasing the significant inflammatory reaction that surrounds the conduit might assist in additional microcanal development. Despite these aspects, our model suggests that the use of such conduits (combined with systemically and locally delivered agents) could potentially be a viable option for men when traditional vasovasostomy is not possible. Additional studies are planned to investigate this possibility further.

CONCLUSIONS

Combined oral sildenafil citrate and locally delivered growth factors increased the number of microcanals present in a biodegradable PDLA conduit used to reconstruct the vas deferens in a rat model.

References