Bone Morphogenetic Protein-7 Improves Renal Fibrosis and Accelerates the Return of Renal Function

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Abstract. A prevention protocol has demonstrated that bone morphogenetic protein-7 (BMP-7) blunted the development of fibrosis in a rat model of unilateral ureteral obstruction. This prevention protocol also preserved, to an extent, renal function. The prevention protocol was extended and a treatment protocol used to examine if BMP-7 was beneficial at limiting fibrosis of the kidney when the BMP-7 was administered during the progression of fibrotic disease. Animals were distributed into four groups. Group 1 received vehicle, group 2 received enalapril (12.5 mg/kg body wt per d), group 3 received BMP-7 (50 or 300 µg/kg), and group 4 received both the enalapril and the high dose of BMP-7. Rats underwent reversible unilateral ureteral obstruction for 3 d, after which the obstruction was relieved. In the treatment protocol, 300 µg/kg BMP-7 was given after the release of obstruction. Seven days after release of the obstruction and the onset of treatment glomerular filtration rate (GFR), renal blood flow, and various histologic indexes of fibrosis were determined. On a consistent basis, BMP-7 treatment alone was found to be slightly but significantly (P < 0.04 to 0.007) better than enalapril alone or in combination with enalapril at decreasing interstitial volume or tubule atrophy. BMP-7 treatment was slightly but not significantly better (P < 0.09) than enalapril at restoring GFR in the prevention protocol. Treatment with BMP-7 significantly boosted GFR (P < 0.01) above that seen with vehicle treatment. These results suggest that BMP-7 treatment is capable of blunting the progression of fibrotic disease and of decreasing interstitial volume. Importantly, a return of renal function is accelerated by BMP-7 treatment. These results suggest that administration of BMP-7 may be an effective treatment to restore or preserve renal histology and renal function in this experimental model of renal disease.

Several kidney diseases of glomerular or nonglomerular origin culminate in expansion and fibrosis of the tubulointerstitial space (1,2). Histologic grading of the fibrosis of the tubulointerstitium is more closely correlated with the loss of renal function than the histologic grading of glomerulosclerosis (3,4). Fibrosis, in general, is an expansion of stromal elements at the expense of highly differentiated parenchymal cells within the tissue (5). The architecture of the kidney entails an exquisite juxtaposition of postglomerular capillaries surrounding the tubules (6–8). This allows for the efficient transfer of fluid and solutes for reabsorption or secretion to maintain homeostasis. In renal disease, the expansion of stromal elements disrupts the kidney architecture and, in all probability, impairs fluid and solute exchange.

Stromal elements that contribute to the derangement of the renal tubulointerstitium and to the eventual development of fibrosis include myofibroblasts, infiltrating monocytes, and an overproduction of extracellular matrix proteins (5). An increase in these stromal elements expands the interstitial volume of the kidney, an event that is readily quantifiable. Another major factor contributing to the loss of renal function during the progression of kidney disease is the loss of parenchymal cells due to apoptosis (9–13). Counterbalancing the loss of epithelial cells is the activation of proliferative pathways in an attempt to recreate tubules within the kidney (14,15). When the ability of tubule epithelial cells to repopulate themselves subsides or is exceeded by apoptotic loss, this results in atrophy of tubules (13). This atrophy is also readily quantifiable.

A contributing mechanism that may account in part for the eventual inhibition of tubule cells to proliferate is the loss or decreased production of several endogenous growth and homeostatic factors in the injured kidney. These factors include, but are not limited to epidermal growth factor (16,17), hepatocyte growth factor (18–20), and bone morphogenetic protein-7 (BMP-7), which is also referred to as osteogenic protein-1 (21). Treatment of experimental animals, with various modalities of renal disease, with some of these factors blunts the development of renal fibrosis and preserves renal function.

In a previous study that used a rat model of unilateral ureteral obstruction (UUO), we found that BMP-7 administration blunted renal fibrosis when given in a prevention protocol (22). In that model, the renal disease was severe and renal function was not measurable in a control group of animals. Administration of BMP-7 partially restored renal function. In this study, we shortened the period of ureteral obstruction from 5 d to 3 d to consistently obtain measures of glomerular...
filtration rate (GFR) and renal blood flow (RBF). The effect of BMP-7 treatment to influence renal architecture and function was determined and was compared with the prevention protocol with the angiotensin-converting enzyme inhibitor, enalapril. More significantly, the effect of BMP-7 treatment in restoring renal function after renal disease had progressed was also determined.

Materials and Methods

Preparation of Animals

Sprague-Dawley rats (approximate weight, 250 g) underwent either a sham operation (ureter manipulated but not ligated) or unilateral ureteral ligation. Two ligatures, 5 mm apart, were placed in the upper two thirds of the ureter (22). The ureter was "protected" during obstruction with a small piece of polyethylene tubing. The suture tied to obstruct the ureter was removed along with the tubing at day 3, relieving the obstruction. The following groups of rats were studied at day 10: sham-operated rats, and rats with release of UUO at 3 d. Sham-operated rats and rats with UUO of 3 d duration had a determination of GFR and renal plasma flow, as assessed by inulin and Paraaminohippurate (PAH) clearance, at day 10.

In a prevention protocol, rats received one of the following doses of BMP-7 10 min before the UUO: soluble BMP-7 (50 μg/kg body wt given intraperitoneally every other day; n = 6); soluble BMP-7 (300 μg/kg given intraperitoneally every other day; n = 3); enalapril (12.5 mg/kg per day) given intraperitoneally (n = 6); vehicle (n = 4); or a combination of 12.5 mg/kg enalapril and 300 μg/kg BMP-7 (n = 5).

In a separate treatment protocol, soluble BMP-7 was administered intraperitoneally every other day (300 μg/kg; n = 4) starting at day 3 during the obstruction. Three separate sham-operated animals were processed simultaneously with this treatment group, along with a new group of vehicle-treated rats (n = 5).

The effects of BMP-7 were compared with placebo (vehicle treatment) and to a positive control (enalapril treatment) known to ameliorate the fibrogenesis resulting from UUO in previous studies from our laboratory and other laboratories (7). The enalapril dose was the same as that used in our previous studies, which had demonstrated the dose effectiveness (7). It is a high dose, much more than that required for modulation of the systemic renin-angiotensin system (22). Studies were performed in a blinded fashion. Treatment solutions were made by an investigator who was not part of the study and were assigned a letter. Investigators were unaware of the contents of the various treatments until after clearance studies and histopathologic grading were completed. The investigator (J.M.) grading the histopathologic slides was also blinded to the treatment groups.

Full-length human BMP-7 cDNA expressed in CHO cells and purified from the medium as a soluble complex was used in the study we present here (22). The complex was composed of the processed mature BMP-7 homodimer noncovalently attached to prodomain protein (referred to as soluble BMP-7). Renal function was determined by measuring GFR with inulin clearances and by estimating RBF by PAH clearances as previously reported (22,23).

Preparation of Kidneys

Rats were anesthetized and catheters were placed in the femoral artery and vein and both ureters. The animals were allowed to waken in a Plexiglas restrainer. Therefore, all clearance studies were performed in animals that were awake. After completing the clearance studies, the animals were killed (methoxyflurane anesthesia), and the kidneys were thoroughly perfused with ice-cold Hanks’ balanced salt solution to remove blood-borne cells. Kidneys were rapidly removed and sliced on a cold glass plate. For histologic studies, 2-mm coronal sections of the Hanks’ balanced salt solution–perfused kidneys were immersed in Histochoice (Amresco, Solon, OH) or in buffered formalin. Kidney sections were embedded in paraffin, and 5-μm sections were analyzed microscopically. We evaluated renal fibrosis by determining interstitial collagen deposition and measuring interstitial volume by immunostaining for type IV collagen.

Quantitation of Fibrosis

Interstitial volume was determined by a point-counting technique (7,22) on tissue sections stained for collagen type IV and was expressed as the mean percentage of grid points lying within the interstitial area in up to five fields in the cortex. A 1-mm² graded ocular grid viewed at ×200 magnification delineated each of these fields.

The degree of fibrosis or the potential for fibrosis in the kidney was determined by scoring the amount of interstitial collagen IV expression. Primary antibodies to collagen type IV (goat polyclonal) were obtained from Southern Biotechnology Association Inc. (Birmingham, AL). We have previously reported the use of these antibodies to assess fibrosis (7,22). The location of the primary antibody was visualized by FITC–anti-goat IgG.

Quantitation of Tubular Atrophy

Kidney sections were stained with antibody to collagen type IV for assessment of tubular basement membranes; tubular atrophy was determined by measuring the tubule diameter. Atrophic tubules were identified by their thickened and sometimes duplicated basement membranes. The diameter of the tubules per field of a ×40 objective was determined, and 10 fields per kidney section were analyzed.

Statistical Analyses

All data were expressed as mean ± SD. Statistical difference was assessed by ANOVA. P < 0.05 was considered to be significant. In the prevention protocol, there was no significant difference in the value between the different doses of BMP-7, so data for these animals were combined.

Results

Interstitial Volume

Obstruction of the ureter causes a progressive increase of the interstitial volume of the kidney cortex. Even after release of the ligation, profibrotic forces still prevail such that the interstitial volume increases from the 7 to 8% found in the kidneys of sham-operated animals to the 17 to 20% found in the obstructed-released kidneys (Table 1). If the obstruction had not been released, the interstitial volume would have increased to approximately 50% by 10 d of continuous obstruction (7,22). In the control, vehicle-treated animals, the interstitial volume averaged 19.8 ± 1.1. This was slightly but significantly decreased by enalapril treatment to 18.1 ± 1.2% (P < 0.05). Treatment with BMP-7 in these rats was more effective, decreasing the interstitial volume to 17.1 ± 0.08% (P < 0.01). Combining enalapril and BMP-7 did not produce any additive or synergistic effects (Table 1.) Although the changes in the interstitial volumes are small, it must be kept in mind that BMP-7 treatment blunted the increase above a baseline of 7% by 21%.
Collagen IV Matrix

In the kidney of normal or sham-operated animals and the contralateral kidneys of rats with UUO, collagen IV is found in the basement membrane surrounding tubules, in capillaries, and in the mesangial matrix. In the setting of fibrosis associated with ureteral obstruction, collagen IV becomes an interstitial matrix protein (7,8,22) in addition to its presence in basement membranes. The collagen IV matrix score is another means of quantifying interstitial fibrosis (7,22). In Figure 1, it

Table 1. Interstitial volume of the kidney cortex

<table>
<thead>
<tr>
<th>Rat</th>
<th>Mean ± SD (%)</th>
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<tbody>
<tr>
<td>Sham operated</td>
<td>7.3 ± 0.3</td>
</tr>
<tr>
<td>Vehicle treated</td>
<td>19.8 ± 1.1</td>
</tr>
<tr>
<td>Enalapril treated</td>
<td>18.1 ± 1.2b</td>
</tr>
<tr>
<td>BMP-7 treated</td>
<td>17.1 ± 0.8c</td>
</tr>
<tr>
<td>Enalapril and BMP-7 treated</td>
<td>17.8 ± 0.8b</td>
</tr>
</tbody>
</table>

a Contralateral kidney interstitial volume varied between 7.6 ± 3% to 8.0 ± 0.3% and did not significantly vary from the value of the kidney of sham-operated animals. BMP-7, bone morphogenetic protein-7.

b \( P < 0.05 \) versus vehicle.

c \( P < 0.01 \) versus vehicle.

Figure 1. Determination of interstitial volume. Sections of kidney cortex were visualized for type IV collagen by immunohistochemistry. An orange grid was superimposed to ascertain the fraction of spots situated over interstitium. BMP-7, bone morphogenetic protein-7. Original magnification, \( \times 400 \).
is seen that collagen IV is present in the interstitial space between the basement membranes surrounding tubules.

Treatment of the animals with enalapril modestly but significantly ($P < 0.05$) decreased the collagen IV matrix score by 15% compared with vehicle. Treatment with BMP-7, significantly decreased the matrix score ($P < 0.005$) by approximately 27% when the baseline score of 0.15 is subtracted from each value (Table 2). A combination of BMP-7 and enalapril also significantly ($P < 0.02$) decreased the collagen IV matrix score by 20%, but this was not different from either treatment alone (Table 2).

Table 2. Collagen IV matrix score of the kidney cortex

<table>
<thead>
<tr>
<th>Rat</th>
<th>Mean ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operated</td>
<td>0.15 ± 0.06</td>
</tr>
<tr>
<td>Vehicle treated</td>
<td>1.88 ± 1.17</td>
</tr>
<tr>
<td>Enalapril treated</td>
<td>1.62 ± 0.08$^b$</td>
</tr>
<tr>
<td>BMP-7 treated</td>
<td>1.44 ± 0.15$^c$</td>
</tr>
<tr>
<td>Enalapril and BMP-7 treated</td>
<td>1.54 ± 0.11$^b$</td>
</tr>
</tbody>
</table>

$^a$ Contralateral kidney collagen IV matrix score varied between 0.13 ± 0.05% to 0.18 ± 0.06% and did not vary significantly from the value of the kidney from sham-operated animals. BMP-7, bone morphogenetic protein-7.

$^b P < 0.05$ versus vehicle.

$^c P < 0.005$ versus vehicle.

Figure 2. Determination of tubule diameter from the same sections as those shown in Figure 1. Lines (orange) spanning basement membrane to basement membrane across tubules were measured. The average tubule width was calculated. BMP-7, bone morphogenetic protein-7. Original magnification, ×400.
**Tubule Diameter**

There is an inexorable loss of tubule epithelial cells through apoptosis during the progression of kidney disease (11,12) resulting in tubule atrophy. This loss may be measured at any interval of time by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assays (11) as the number of cells undergoing apoptosis at that point in time. Another means by which tubule atrophy may be determined is by measuring tubule diameter, which would be an index of all apoptotic cell loss up to that point in time when the kidney biopsy is obtained. Figure 2 depicts the same fields of renal cortex seen in Figure 1 but with lines measuring the tubule diameter.

Two issues emerge from this determination. First, the average tubule diameter in the section of kidney with an obstructed ureter is less than that of kidneys of sham-operated rats. Second, the number of tubules in the field of the kidney with an obstructed ureter (Figure 2) is less than that found in the section of the kidney obtained from a sham-operated rats (Figure 2). In all sections of kidney with an obstructed ureter, whether treated with vehicle, enalapril, BMP-7, or both, there were swollen tubules. An elegant study by Truong et al. (24) showed that a measure of tubule diameter provides a dynamic measure of apoptotic loss of individual tubule cells in this model of renal disease. When the swollen tubules are accounted for, it is apparent that all treatments preserve tubule diameter (Table 3, Figure 2). The preservation of tubule diameter is significantly greater ($P < 0.04$) in the BMP-7-treated group than in groups with other treatments (Table 3).

**Renal Function Studies: Prevention Protocol**

The presumption that is made is that detrimental changes in the histologic appearance of the kidney are reflected in concomitant decreases in renal function. Conversely, ameliorative changes in histologic appearance should translate into better renal function. Table 4 shows that GFR was decreased by about half, whereas RBF was decreased by approximately 20% in the obstructed-released kidney of vehicle-treated rats compared with sham-operated rats. The reduction in GFR was significant ($P < 0.02$). Treatment with enalapril alone ($P < 0.07$) tended toward but was not significantly different from vehicle treatment. Animals that had been treated with BMP-7 had a significant recovery of GFR compared with vehicle treatment ($P < 0.01$) and were statistically indistinguishable ($P > 0.10$) from sham-operated rats (Table 4). Treatment with a combination of enalapril and BMP-7 was on average as effective, but because of the wider SD, it was not statistically different from treatment with vehicle ($P < 0.12$). In these experiments, RBF was not significantly different between the groups of animals. Significantly, all animals had renal function after release of the obstruction that was measurable at day 10 or 7 d after relief of the obstruction of 3 d duration.

**Renal Function Studies: Treatment Protocol**

The prevention protocol clearly suggests that BMP-7 given at the initiation of and throughout the 10-d period of experimental renal disease preserves renal function. In the treatment protocol, the BMP-7 was withheld until the time that the obstruction was surgically corrected. After 7 d of release with concomitant treatment, it was found that BMP-7 significantly restored or accelerated the return of renal function (Figure 3). This treatment protocol included three new sham-operated and five new vehicle-treated rats, along with the four BMP-7–treated animals. There was a significant ($P < 0.001$) restoration of renal function measured as GFR in the BMP-7 treatment group compared with vehicle treatment. Treatment with BMP-7 increased GFR more than fourfold above vehicle treatment such that 70% of GFR was restored.

This significant preservation of GFR was accompanied by a significant decrease in the interstitial volume ($P < 0.001$) in the BMP-7 treatment group compared with the vehicle treatment group (Figure 3). In this group of animals, the cortical interstitial volume of the obstructed-released kidney was $25.0 \pm 2.3\%$. This was reduced to $15.4 \pm 2.1\%$ by BMP-7 treatment, which was modestly but significantly ($P < 0.025$) elevated above the cortical interstitial volume of the kidney in sham-operated rats.

**Discussion**

The study we present here adds to our previous findings (22) demonstrating that BMP-7 may be used in treatment protocols to improve renal architecture during the course of renal disease. More important, BMP-7 accelerated the recovery of renal function in this treatment regimen. Our previous study (22) involved only prevention protocols and was consistent with the present prevention protocol study in which all animals had renal function.

On a consistent basis, BMP-7 treatment alone appears to be somewhat better than enalapril treatment alone or a combination of enalapril and BMP-7 in the prevention study. With respect to the histologic indexes of renal fibrosis, this difference between BMP-7 and enalapril was significant, varying from $P < 0.04$ for tubule diameter recovery to $P < 0.007$ for the interstitial volume. The greater recovery of GFR attributable to BMP-7 treatment over enalapril in the prevention treatment approached, but did not achieve, significance ($P < 0.09$). We currently do not know the extent of involvement of

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**Table 3. Tubule diameter**

<table>
<thead>
<tr>
<th>Rat</th>
<th>Diameter, Mean ± SD ($\mu$m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operated</td>
<td>71 ± 4</td>
</tr>
<tr>
<td>Vehicle treated</td>
<td>53 ± 6$^b$</td>
</tr>
<tr>
<td>Enalapril treated</td>
<td>59 ± 5$^c$</td>
</tr>
<tr>
<td>BMP-7 treated</td>
<td>65 ± 4$^d$</td>
</tr>
<tr>
<td>Enalapril and BMP-7</td>
<td>59 ± 5$^c$</td>
</tr>
</tbody>
</table>

$^a$ The tubule diameter of the contralateral unobstructed kidneys were not significantly different from the value of the kidney of sham-operated animals. BMP-7, bone morphogenetic protein-7.

$^b$ $P < 0.0005$ versus sham.

$^c$ $P < 0.01$ versus sham.

$^d$ $P < 0.05$ versus sham; $P < 0.002$ versus vehicle; $P < 0.04$ versus enalapril or enalapril and BMP-7.
BMP-7 in various molecular biologic or cell biologic pathways and cannot speculate why the combination of BMP-7 and enalapril is not as effective as BMP-7 alone. Enalapril, an angiotensin-converting enzyme inhibitor, has multiple pharmacologic actions. It is possible that BMP-7 may blunt an action of angiotensin-converting enzyme inhibition such as the elevation of nitric oxide, which we have found to be beneficial in ameliorating tubulointerstitial fibrosis in this model of renal disease (25).

BMP-7 is a member of the transforming growth factor beta (TGF-β) superfamily and is an important morphogen for kidney development (26–28). BMP-7 continues to be expressed in the adult mammalian kidney (29), and its expression appears to be downregulated in both kidneys, even in unilateral disease models (30). In preliminary studies that used the rodent model of UUO, it was found that BMP-7 mRNA levels are severely depressed in the kidney with an obstructed ureter. It is possible that our provision of exogenous BMP-7 replaces the endogenous BMP-7 that is lost as a result of renal disease. This suggests that some mechanism exists within the kidneys that regulates BMP-7 expression. The existence of such a mechanism and its nature remain to be determined.

BMP-7 affects cell signaling by binding to transmembrane serine threonine kinase receptor complexes (31), which are present throughout the kidney (32). This binding to the cell surface receptor activates an intracellular protein referred to as Smad6, which antagonizes the action of TGF-β (31,33,34). The pathophysiology of the tubulointerstitial fibrosis of obstructive nephropathy is driven in part by an increase in TGF-β synthesis within the affected kidney (3,4,7,25). Therefore, activation of existing Smad6 or an increase in Smad6 synthesis by BMP-7 (34) may provide a mechanism by which this growth factor counteracts profibrotic forces within the kidney. We had shown earlier (22) that BMP-7 reduced the number of TUNEL-positive cells undergoing apoptosis in this model of renal disease. It appears that BMP-7 treatment stabilizes renal epithelial cells and prevents their loss, thereby preserving eventual renal function.

In our previous investigation (22) and the study we present here, it was concluded that BMP-7 protects the kidney against

**Table 4. Glomerular filtration rate (GFR) and renal blood flow (RBF)**

<table>
<thead>
<tr>
<th>Rat</th>
<th>GFR (ml · min⁻¹ · 100 g body wt⁻¹)</th>
<th>RBF (ml · min⁻¹ · 100 g body wt⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operated</td>
<td>0.34 ± 0.07</td>
<td>1.02 ± 0.06</td>
</tr>
<tr>
<td>Vehicle treated</td>
<td>0.16 ± 0.02b</td>
<td>0.83 ± 0.22</td>
</tr>
<tr>
<td>Enalapril treated</td>
<td>0.21 ± 0.05b</td>
<td>0.92 ± 0.23</td>
</tr>
<tr>
<td>BMP-7 treated</td>
<td>0.26 ± 0.06c</td>
<td>0.93 ± 0.24</td>
</tr>
<tr>
<td>Enalapril and BMP-7 treated</td>
<td>0.27 ± 0.13</td>
<td>1.01 ± 0.49</td>
</tr>
</tbody>
</table>

*a The GFR of the contralateral unobstructed kidneys varied between 0.35 ± 0.08 and 0.47 ± 0.13. This was not significantly different from the GFR of the kidney of sham-operated animals (P < 0.08 to 0.75). The RBF of the contralateral unobstructed kidneys varied between 1.17 ± 0.28 to 1.48 ± 0.19. BMP-7, bone morphogenetic protein-7.

*b P < 0.02 versus sham.

*c P < 0.01 versus vehicle and P > 0.10 versus sham.

**Figure 3.** Renal function (glomerular filtration rate; GFR) and renal cortical interstitial volume (Vv_int) of rats in a bone morphogenetic protein-7 (BMP-7) treatment protocol. GFR was measured as milliliters per minute per 100 g of body wt; Vv_int was measured as the percentage of cortical volume occupied by interstitium. CK, contralateral unobstructed kidney; OB, kidney with an unobstructed ureter.
the development of fibrosis when the drug is initiated at the time that the ureteral obstruction is established. Here, we also clearly show that BMP-7 treatment is capable of slowing, halting, and possibly reversing molecular and cellular events that lead to loss of renal function when given after renal disease processes are initiated. BMP-7 appears to be an important renal homeostatic factor as well as being an important renal morphogen. However, further experiments in animals are needed before recommending the use of this compound in patients with progressive renal disease.

Acknowledgments

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References