

# Treatment with Metalloproteinase Inhibitor XL784 Prevents the Development of Glomerulosclerosis in Dahl SS Hypertensive rat



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## ABSTRACT

Dahl salt-sensitive (Dahl SS) rats are a well established model of hypertension-induced proteinuria and glomerulosclerosis. The present experiments examined the effects of XL784, an inhibitor of ADAM-10, -17 and matrix metalloproteinases (MMP)-2, -3, -8, -9 and -13, on the development of glomerulosclerosis in Dahl SS rats. When fed a high salt diet (4% for 5 weeks), control Dahl SS rats develop increased blood pressure (>180 mmHg) and severe global glomerulosclerosis. Treatment with XL784 at doses of 50, 125 or 250 mg/kg/day at the start of the high salt diet regimen resulted in a dose-dependent decrease in proteinuria and the degree of glomerular disease. To evaluate the effects of XL784 on pre-existing renal disease, Dahl SS rats were fed a 4.0% high salt diet for 5 weeks and then treated with either 50 mg/kg XL784, ACE inhibitor lisinopril (LIS) and ARB losartan (LST) (20 mg/kg) or a combination of XL784, LIS and LST for an additional 5 weeks while being maintained on the high salt diet. The increase in proteinuria was prevented in rats treated with either 50 mg/kg/day XL784 or LIS and LST. Proteinuria fell to  $46 \pm 3$  mg/day in rats treated with the combination of XL784, LIS, and LST. This value is comparable to that seen in age matched non-hypertensive Dahl SS rats fed a low salt diet. The degree of glomerulosclerosis and renal interstitial fibrosis was significantly reduced by about the same extent with XL784 alone or the inhibitors of the renin-angiotensin system. Combined treatment with XL784, LIS and LST completely reversed the degree of glomerular injury and renal interstitial fibrosis to the levels seen in age matched Dahl SS rats maintained on a low salt diet throughout the study. These studies indicate that metalloproteinase inhibitor XL784 alone or in combination with ACE inhibitor LIS and ARB LST can prevent the development and even reverse preexisting glomerulosclerosis and renal interstitial fibrosis.

## INTRODUCTION

\* Dahl salt-sensitive (Dahl SS) rats are a well established model of hypertension-induced renal injury.

\* Up-regulation of ADAM ADAM-10, -17 and matrix metalloproteinases (MMP)-2, -3, -8, -9 and -13 has been implicated in the development of renal disease. XL784 is an inhibitor of these pathways.

## SPECIFIC AIM

To test the effectiveness of XL784 versus blockade of the RAS in preventing hypertension-induced end organ damage in Dahl SS rats

## EXPERIMENTAL PROTOCOL

\* 6 week old Dahl S rats were fed a high salt diet (4% NaCl) for 5 weeks to induced the development of hypertension and renal disease.

\* The animals were then treated with:  
 1) vehicle  
 2) LIS and LST -20 mg/kg/day each  
 3) XL784- 50 mg/kg/day  
 4) LIS and LST plus XL784

\* Proteinuria was followed weekly for an additional 4 weeks while the rats remained on the high salt diet.  
 \* After four weeks an indwelling femoral artery catheter was implanted and arterial blood pressure measured on 3 consecutive days.  
 \* After blood pressure was measurement, the rats were euthanized and a blood sample for clinical chemistry and the heart, liver, and kidneys for histological evaluation.

## RESULTS

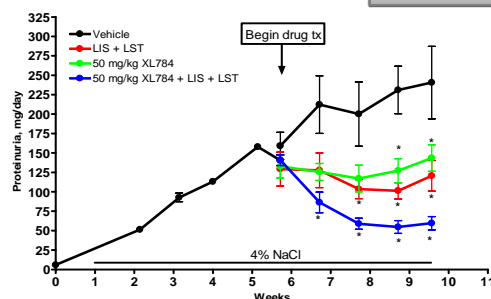


Figure 1. Effects of XL784 and blockade of the RAS on proteinuria in Dahl SS rats

Animals were randomly assigned to one of four treatment groups on day 40; treatment began on day 41. By study day 61, all treatment groups had lower proteinuria than control animals. \*P < 0.05 vs. vehicle. N = 12 rats per group.

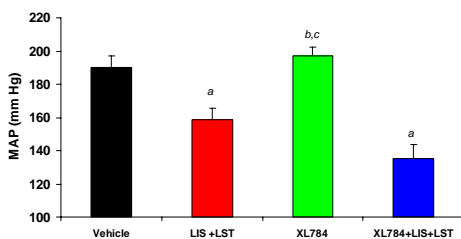


Figure 2. Effects of XL784 versus inhibitors of the RAS on mean arterial pressure in Dahl SS rats

Inhibition of the renin-angiotensin system with the ACE inhibitor LIS and the AT1 receptor antagonist LST decreased mean arterial pressure in Dahl SS rats. The decrease in blood pressure was ~16% with LIS and LST while the decrease with XL784, LIS, and LST in combination was ~29%. Treatment of Dahl SS rats with preexisting disease with XL784 alone did not lower blood pressure. aP < 0.05 vs. vehicle. bP < 0.05 vs. LIS + LST. cP < 0.05 vs. XL784 + LIS + LST

| Analyte*             | Vehicle         | LIS + LST                 | 50 mg/kg/day XL784           | 50 mg/kg/day XL784 + LIS + LST |
|----------------------|-----------------|---------------------------|------------------------------|--------------------------------|
| Glucose, mg/dL       | 147.2 ± 2.5     | 149.8 ± 6.0               | 151.8 ± 2.6                  | 150.7 ± 3.6                    |
| AST, U/L             | 163.9 ± 16.2    | 342.0 ± 43.3 <sup>b</sup> | 182.5 ± 29.4 <sup>a</sup>    | 229.3 ± 53.8                   |
| ALT, U/L             | 52.90 ± 3.59    | 63.00 ± 5.73              | 53.90 ± 5.05                 | 53.56 ± 8.10                   |
| ALP, U/L             | 101.1 ± 5.03    | 91.89 ± 3.39              | 100.0 ± 5.13                 | 106.3 ± 15.8                   |
| Cholesterol, mg/dL   | 119.3 ± 11.9    | 103.8 ± 5.11              | 108.4 ± 6.96                 | 88.44 ± 4.96 <sup>b</sup>      |
| BUN, mg/dL           | 21.10 ± 1.61    | 19.33 ± 1.50              | 22.90 ± 0.781                | 22.33 ± 2.67                   |
| Creatinine, mg/dL    | 0.2700 ± 0.0260 | 0.2222 ± 0.0147           | 0.2800 ± 0.0249              | 0.2333 ± 0.0333                |
| Phosphate, mg/dL     | 7.020 ± 0.245   | 7.122 ± 0.249             | 7.840 ± 0.183                | 8.500 ± 0.694 <sup>a,c</sup>   |
| Calcium, mg/dL       | 10.57 ± 0.0857  | 10.53 ± 0.253             | 10.39 ± 0.0767               | 10.17 ± 0.154                  |
| Sodium, mM           | 143.5 ± 0.500   | 144.1 ± 0.735             | 142.9 ± 0.458                | 143.4 ± 0.604                  |
| Potassium, mM        | 4.960 ± 0.240   | 6.975 ± 1.875             | 4.98 ± 0.313                 | 6.100 ± 0.286                  |
| Chloride, mM         | 100.6 ± 0.499   | 101.0 ± 0.333             | 103.7 ± 0.761 <sup>a,c</sup> | 102.8 ± 1.86 <sup>a,c</sup>    |
| LDH, U/L             | 218.9 ± 14.27   | 252.6 ± 35.2              | 333.3 ± 94.02                | 229.1 ± 36.5                   |
| Triglycerides, mg/dL | 170.6 ± 15.8    | 153.9 ± 13.4              | 109.5 ± 16.5 <sup>b</sup>    | 72.44 ± 9.43 <sup>a,c</sup>    |
| CK, U/L              | 145.8 ± 33.0    | 423.2 ± 265               | 234.1 ± 67.2                 | 260.3 ± 36.8 <sup>a</sup>      |
| SDH, U/L             | 2.630 ± 0.342   | 2.089 ± 0.0889            | 2.240 ± 0.171                | 2.000 ± 0.000                  |

Table 1. Comparison of the effects of XL784 versus blockade of the RAS on various indices of clinical chemistry in Dahl SS rats  
 The combination of XL784 with LIS and LST treatment lowered serum cholesterol and triglycerides. This group also displayed elevations in serum phosphate, chloride, and CK. XL784 alone lowered triglycerides and AST while also has an elevated chloride compared to vehicles. While treatment with LIS and LST only increased AST.

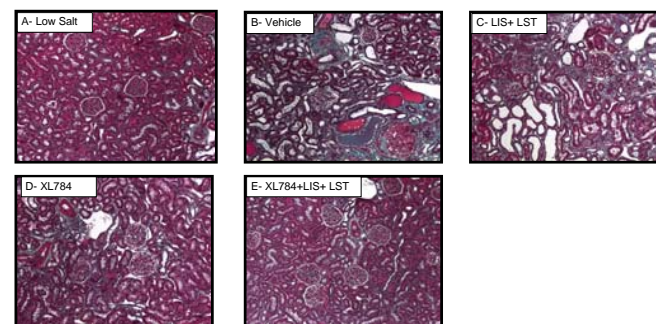


Figure 3. Effect of treatment of XL784 on glomerulosclerosis in Dahl SS rats

Left kidneys were stained with Masson's trichrome stain. Glomeruli were scored for pathological changes in blinded fashion on a 0-4 scale. 0 = no sclerosis, all capillaries are open; 1 = slight sclerosis, < 25% of capillaries are occluded; 2 = mild sclerosis, < 50% of capillaries are occluded; 3 = moderate sclerosis, < 75% of capillaries are occluded, some structural defects visible; 4 = severe sclerosis with significant structural defects. In vehicle treated animals, they exhibited a high degree of mesangial expansion and occlusion on the capillaries. All treatments protected against the glomerulosclerosis that was observed in vehicle-treated animals and XL784 was more effective than LIS + LST.

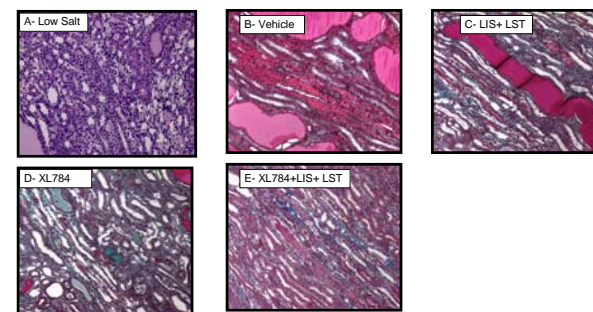


Figure 4. Effect of treatment of XL784 on the outer medulla in Dahl SS rats

Kidneys were scored (0-3 scale) for vasa recta fibrosis as follows: 0 = all capillaries are open; 1 = some blue staining but capillaries are open; 2 = blue staining and some occluded capillaries but many open capillaries remain; 3 = blue staining and few or no open capillaries. The number of protein casts was counted using a proprietary image analysis software program. In vehicle Dahl SS animals the outer medulla and the vasa recta bundles were completely fibrosed and the capillaries were completely occluded. The thick ascending limbs were filled with protein. In contrast, animals treated with XL784 alone or in combination with LIS+LST reduced the protein casts in the outer medulla while the patency of the capillaries.

| Treatment              | Renal injury index          |                             |                           |
|------------------------|-----------------------------|-----------------------------|---------------------------|
|                        | Glomerulosclerosis          | Vasa recta fibrosis         | Protein casts             |
| Vehicle (n = 11)       | 2.53 ± 0.070                | 1.23 ± 0.11                 | 17.9 ± 0.40               |
| LIS + LST (n = 11)     | 2.08 ± 0.050 <sup>a</sup>   | 0.864 ± 0.079 <sup>a</sup>  | 5.47 ± 1.1 <sup>a</sup>   |
| XL784 (n = 11)         | 1.76 ± 0.072 <sup>a,b</sup> | 1.20 ± 0.023 <sup>a,c</sup> | 12.7 ± 2.0 <sup>a,c</sup> |
| XL784+LIS+LST (n = 10) | 1.56 ± 0.078 <sup>a,b</sup> | 0.675 ± 0.063 <sup>a</sup>  | 2.60 ± 1.7 <sup>a</sup>   |

Table 2. Comparison of the effects of XL784 versus blockade of the RAS on various indices of renal injury  
 All of the drug-treated rats exhibited less glomerulosclerosis than did the vehicle-treated rats. In addition, XL784 alone or in combination with LIS and LST was more effective than LIS and LST in inhibiting glomerulosclerosis.

## CONCLUSION

1. XL784 prevents the progression of proteinuria and glomerular injury in Dahl SS rats with established hypertension and preexisting renal disease without reducing mean arterial pressure. It is at least as effective as a combination of an ACE inhibitor and an AT1 blocker.
  2. There appears to be a consistent XL784 effect to moderately lower serum cholesterol and triglycerides.
  3. XL784 in combination with lisinopril and losartan was more effective than the individual treatments. Glomerular injury was reduced to levels typically seen in animals fed a low salt diet suggesting that this treatment has the potential to reverse preexisting glomerular injury.
- XL784 in combination with lisinopril and losartan shows great potential for the prevention and even the reversal of hypertension induced glomerulosclerosis.