AT₁ blockade during lactation as a model of chronic nephropathy: mechanisms of renal injury

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Machado FG, Poppi EP, Fanelli C, Malheiros DM, Zatz R, Fujihara CK. AT₁ blockade during lactation as a model of chronic nephropathy: mechanisms of renal injury. Am J Physiol Renal Physiol 294: F1345–F1353, 2008. First published April 9, 2008; doi:10.1152/ajprenal.00020.2008.— Suppression of the renin-angiotensin system during lactation causes irreversible renal structural changes. In this study we investigated 1) the time course and the mechanisms underlying the chronic kidney disease caused by administration of the AT₁ receptor blocker losartan during lactation, and 2) whether this untoward effect can be used to engender a new model of chronic kidney disease. Male Munich-Wistar pups were divided into two groups: C, whose mothers were untreated, and L₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄₄¢F1345 The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: R. Zatz, Laboratório de Fisiopatologia Renal, Av. Dr. Arnaldo, 455, 3-s/3342, 01246-903 São Paulo, Brazil (e-mail: rzatz@usp.br).

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**RESULTS**

**Functional studies.** Functional parameters measured in rats at 3 mo of age are shown in Table 1. No difference in body weight was observed between groups. In agreement with ear-
Table 1. Renal function study at 3 mo of age

<table>
<thead>
<tr>
<th></th>
<th>C (n = 5)</th>
<th>Lact (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>323±1</td>
<td>285±1</td>
</tr>
<tr>
<td>NN, ×10³</td>
<td>27±1</td>
<td>18±1*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>108±2</td>
<td>104±1</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>1.42±0.07</td>
<td>0.94±0.06*</td>
</tr>
<tr>
<td>RPF, ml/min</td>
<td>4.13±0.24</td>
<td>2.77±0.27*</td>
</tr>
<tr>
<td>RVR, mmHg·ml⁻¹</td>
<td>14.3±0.7</td>
<td>20.9±2.3*</td>
</tr>
<tr>
<td>Pvac, mmHg</td>
<td>53±1</td>
<td>63±2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; BW, body weight; NN, nephron number (per 1 kidney); MAP, mean arterial pressure; GFR, glomerular filtration rate; RPF, renal plasma flow; RVR, renal vascular resistance; Pvac, glomerular hydraulic pressure. *P < 0.05 vs. C.

Long-term studies. Renal and functional parameters obtained at 3 and 10 mo of age are represented in Table 2. Body weight was similar between C and Lact rats at 3 mo and also at 10 mo of age. Left kidney weight did not differ between groups but increased with time in C rats. No differences between groups or an effect of time were observed regarding PNa or PK. Scr was similar between groups but increased with time in C rats. No differences between groups or an effect of time were observed regarding Vol., Uosm, or Screat. PNa or PK were similar between groups at 3 mo of age but increased with time in C rats. No statistically significant difference was observed regarding urine flow at 3 mo of age. Urine volume in Lact rats at 3 mo of age was 1.18 ± 0.27 (P < 0.05 LLact vs. C). Accordingly, urine osmolality was 30% lower in Lact than in C rats at 3 mo and was reduced by 46%. Although Lact rats were normotensive, PGC was 10 mmHg above this in group (P < 0.05).

The time course of TCP is described in Fig. 1A. As in the functional studies, Lact rats were normotensive at 3 mo of age. TCP remained at normal levels at 6 mo of age. However, TCP rose significantly at 10 mo in Lact rats only. No statistically significant difference was observed regarding urine flow at 3 mo of age. The time course of the urine albumin excretion rate. Albuminuria was always higher in Lact rats and increased by 46%. Although Lact rats were normotensive, PGC was similar between Lact and C. However, GFR and RPF were reduced by nearly one-third in Lact rats, whereas SCR was increased by 46%. Although Lact rats were normotensive, PGC was similar between Lact and C. However, GFR and RPF were reduced by nearly one-third in Lact rats, whereas SCR was increased by 46%. Although Lact rats were normotensive, PGC was 10 mmHg above this in group (P < 0.05).

The analysis of renal structural injury and inflammation is detailed in Figs. 3 and 4. Glomerular segmental sclerotic lesions, which were infrequent in Lact rats at 3 mo of age, were abundant at 10 mo of age (Figs. 3A and 4A). Expansion and inflammation of the interstitial tissue were already observed at 3 mo in Lact rats and were drastically increased at 10 mo of age (Figs. 3B and 4B). Infiltration of the renal interstitium by macrophages (Figs. 3C and 4C) and by cells staining positively for ANG II (Figs. 3D and 4D) followed a similar pattern, with intensity roughly proportional to that of interstitial expansion.

A statistical analysis of the glomerular dimensions is shown in Fig. 5. In rats at 3 mo of age, glomerular areas followed a pattern not significantly different from a Gaussian distribution in normal controls (Fig. 5, A and B), the calculated glomerular volume averaging 0.90 ± 0.03 × 10⁶ μm³. In Lact rats, a large number of unusually small glomerular profiles were observed. The appearance of these glomeruli was inconspicuous (Fig. 5A), whereas in several of them the hilum was clearly visible (Fig. 5A, inset), ruling out the possibility that these profiles represented solely glomeruli sectioned close to a pole. The presence of these small glomeruli skewed the frequency distribution of glomerular areas to the left, promoting a significant deviation from normality. Accordingly, the percentage of profiles with areas <3,000 μm² was 18 ± 3% in Lact compared with only 6 ± 1% in C rats (P < 0.05). The mean glomerular volume in Lact rats at 3 mo of age was 1.18 ± 0.06 × 10⁶ μm³ (P < 0.05 vs. control). When the profiles with areas under 3,000 μm² were excluded from the calculation, the average glomerular volume for Lact rats was even higher (1.50 ± 0.08 × 10⁹ μm³), further underlining the occurrence of glomerular compensatory growth in Lact rats. In agreement with this concept, the fraction of glomerular profiles with areas exceed-

Table 2. Renal functional and systemic parameters at 3 and 10 mo of age

<table>
<thead>
<tr>
<th></th>
<th>C (n = 10)</th>
<th>Lact (n = 10)</th>
<th>C (n = 13)</th>
<th>Lact (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>278±9</td>
<td>264±10</td>
<td>417±7†</td>
<td>393±11†</td>
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<tr>
<td>LKW, g</td>
<td>1.49±0.06</td>
<td>1.67±0.05</td>
<td>1.97±0.05†</td>
<td>1.82±0.06</td>
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<tr>
<td>PNa, mmol/l</td>
<td>139±2</td>
<td>141±2</td>
<td>142±2</td>
<td>140±1</td>
</tr>
<tr>
<td>PK, mmol/l</td>
<td>3.7±0.1</td>
<td>3.9±0.2</td>
<td>4.3±0.2</td>
<td>4.3±0.2</td>
</tr>
<tr>
<td>Scr, mg/dl</td>
<td>0.64±0.11</td>
<td>0.70±0.09</td>
<td>0.68±0.04</td>
<td>0.94±0.06†</td>
</tr>
<tr>
<td>Vt, ml/min</td>
<td>23±2.2</td>
<td>31±1.5</td>
<td>29±1.7</td>
<td>64±7.1†</td>
</tr>
<tr>
<td>Uosm, mosmol/kgH₂O</td>
<td>1,210±116</td>
<td>921±45*</td>
<td>1,111±83</td>
<td>543±58†</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; LKW, left kidney weight; PNa, plasma sodium concentration; PK, plasma potassium concentration; Scr, serum creatinine concentration; Vt, ml/min; Uosm, urine osmolality. *P < 0.05 vs. C. †P < 0.05 vs. respective 3-mo value.
neighboring 15,000 μm² was 17 ± 3% in Lₐct compared with only 1 ± 1% in C rats (P < 0.05). At 10 mo of age, the distribution of glomerular areas in normal controls was skewed to the right, indicating that a substantial number of glomeruli underwent hypertrophy. Indeed, the mean glomerular volume (excluding the profiles measuring <3,000 μm²) was increased at this time in C (1.56 ± 0.05 × 10⁶ μm³) compared with the value obtained at 3 mo of age (P < 0.05), whereas the percentage of glomerular profiles with areas >15,000 μm² was 15 ± 2% (P < 0.05 vs. the corresponding 3-mo value). In Lₐct rats, the distribution of areas was also shifted to the right, whereas the mean glomerular volume (again excluding profiles with areas <3,000 μm²) was 2.03 ± 0.08 × 10⁶ μm³ (P < 0.05 vs. 10-mo C and 3-mo Lₐct) and the proportion of profiles >15,000 μm² was 35 ± 2% (P < 0.05 vs. 10-mo C and 3-mo Lₐct), indicating additional glomerular hypertrophy in these rats. The percentage of small glomeruli (profile area <3,000 μm²) remained constant with time in Lₐct rats (18 ± 2%, P > 0.05 vs. the 3-mo value), suggesting that this population of hypotrophic glomeruli is relatively stable.

DISCUSSION

As described previously, blockade of the AT-1R during lactation resulted in a 30% reduction in the number of nephrons, confirming previous reports (29, 32, 35), although some discordant findings have been described (19), and corroborating the concept that the presence of ANG II is essential for adequate nephron maturation (8, 32). The molecular mediators and the cellular signaling pathways that may be responsible for this salutary action of ANG II on nephrogenesis are presently unclear, although evidence has been reported that activation of insulin-like growth factor (IGF) and of its receptor may play a crucial role (24).

Confirming previous reports (2, 7, 9, 32, 35), Lₐct rats developed severe and progressive renal functional and structural changes, that were nevertheless associated with zero mortality, making it possible to follow these rats until very advanced stages. This behavior was remarkably homogeneous: at 3 mo of age, all rats in the Lₐct group were diseased, and low coefficients of variation were observed for parameters such as albuminuria and TCP. It must be emphasized that in the present study the teratogenic effect of losartan was obtained by offering the drug to the dams in the drinking water, taking advantage of the fact that losartan easily crosses the blood-milk barrier (31). It was thus possible to obviate the need for individual intraperitoneal injections, utilized in many previous studies.

At 3 mo of age, GFR was diminished by 30% in Lₐct rats compared with C rats. This finding is in agreement with most previous reports (7, 8, 29, 35), despite a few discordant observations (17). Since the reduction in GFR and in RPF was proportional to the reduction in the number of nephrons, it is tempting to conclude that no compensatory hyperfiltration or hyperperfusion, such as observed in rats with uninephrectomy or 5/6 renal ablation (3, 33), occurs in this model. However, it must be noted that a substantial fraction of glomeruli in Lₐct rats was found to be hypotrophic, a proportion likely underestimated since, unlike normal glomeruli, hypotrophic glomeruli are easily overlooked if sectioned close to one of the poles. Since these hypotrophic glomeruli, which probably result from nephron malformation, are expected to contribute little to the overall GFR and may even have lost their connection with the respective tubules (4), some compensatory hyperfiltration must have occurred in the remaining nephrons. On the other hand, the finding that a substantial number of glomeruli in Lₐct rats

Fig. 1. Time course of tail-cuff pressure (TCP: A) and urine albumin excretion rate (Ualb/V; B) in control rats (C; n = 13) and in rats treated with losartan during lactation (Lₐct; n = 13). aP < 0.05 vs. respective control value. bP < 0.05 vs. respective 3-mo value. cP < 0.05 10-mo vs. respective control value. dP < 0.05 vs. respective 3-mo value.

Fig. 2. Selectivity index of proteinuria (SI; left) and expression of the zonula occludens-1 (ZO-1) protein (right) in control (open bars) and Lₐct (filled bars). *P < 0.05 Lₐct vs. C.

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exhibited mean cross-sectional areas in excess of 15,000 μm² (the frequency of such glomeruli was negligible in controls) indicates that compensatory glomerular hypertrophy also occurred in these animals.

The intimate mechanisms underlying the compensatory increase in single-nephron GFR in L_Lact rats are not entirely clear. Part of the compensatory response likely involved an elevation of PGC, at least at 3 mo of age (unfortunately, the severe deformation of the renal surface due to interstitial expansion/inflammation precluded the determination of PGC by micropuncture at 10 mo of age). Since systemic hypertension was absent in these rats at the time the functional studies were performed, glomerular hypertension, at least at this phase, must have resulted from afferent arteriolar dilatation and/or efferent arteriolar vasoconstriction. It is unclear whether this arteriolar dysfunction is merely part of a compensatory response or whether arteriolar malfunctioning in adult life constitutes another ill effect of losartan administration during nephrogenesis. It should be noted that the number of litters (21) and the number of functional observations (5/group) were both relatively small and, although differences between groups were almost invariably statistically significant, the present functional measurements inevitably carry some degree of uncertainty. Thus the results of the present functional studies should be interpreted with caution, and definitive conclusions in this regard will have to await confirmation by future studies.

L_Lact rats developed several aspects of chronic glomerular structural and functional injury. Albuminuria was already evident at 3 mo of age and exhibited a clearly progressive nature, reaching levels one order of magnitude higher than in C rats at 10 mo of age. Albuminuria was associated with limitation of the size-selective properties of the glomerular walls, as assessed by the increase in a selectivity index, already observed at 3 mo of age. However, since the selectivity index was not further increased at 10 mo of age, the aggravation of albuminuria observed at this time must have resulted from factors in addition to a size defect, such as rarefaction of the negative surface charge of the glomerular walls. The reduced expression of the ZO-1 molecule suggests that podocyte injury may explain, at least in part, the development of albuminuria in L_Lact rats. Since podocytes are responsible for the synthesis of part of the negatively charged molecules that contribute to limit the passage of polyanions through the glomerular walls, podocyte damage can explain the development of proteinuria by a
combined defect in the size- and charge-selective properties of the glomerular wall.

Although albuminuria was already present at 3 mo of age, glomerular injury at this time was only mild, although significant, in L_{Lact} rats compared with C rats. Glomerulosclerosis was much more pronounced at 10 mo of age, the GSI reaching levels over 50-fold higher, whereas serum creatinine was significantly elevated, compared with age-matched C rats. Although the exact mechanisms leading to the development of glomerular injury in this model are not entirely clear, an obvious possibility is that the maladaptive glomerular hypertension and hypertrophy represented important pathogenic factors. Glomerular hypertension and/or hypertrophy have been postulated to initiate and maintain progressive glomerular injury in several experimental models (22, 36) by promoting mechanical stretch to the glomerular walls according to La Place’s law (26) and, as a consequence, podocyte injury with formation of sinechiae with Bowman’s capsule (20, 30), mesangial expansion (25), and infiltration by inflammatory cells (27). This process tends to acquire a progressive nature as a growing number of glomeruli drop out due to severe sclerosis, and an additional burden is imposed on the remaining nephrons. Absence of early renal hemodynamic changes may be one of the reasons female rats fail to develop progressive nephropathy after neonatal exposure to ANG II receptor blockers or angiotensin-converting enzyme inhibitors (17).

A progressive increase in the fractional cortical interstitial area was, along with glomerular injury, a prominent feature of the nephropathy that developed in the L_{Lact} group. Interstitial expansion was associated with clear signs of chronic inflammation, such as interstitial fibrosis and infiltration by mononuclear cells, a large fraction of which were macrophages. Curiously, many of these inflammatory cells stained positively for ANG II, suggesting that, although its presence during nephrogenesis is essential for adequate nephron maturation, ANG II exerts in this model a similar proinflammatory role as in other experimental models of progressive nephropathy (6). The pathogenesis of interstitial expansion/inflammation in this model is unclear. Interstitial injury could be a direct consequence of glomerular injury due to propagation of tuft inflammation through sinechiae with Bowman’s capsule and direct passage of circulating inflammatory mediators to the periglomerular interstitium (14). In addition, the exaggerated filtration of proteins due to impairment of the glomerular barrier might induce the synthesis of cytokines, chemokines, and growth factors by proximal tubular cells in association with augmented protein endocytosis (1). However, interstitial expansion and inflammation were already evident in L_{Lact} rats at 3 mo of age, when albuminuria and glomerular injury were still mild. Therefore, the development of marked interstitial injury in L_{Lact} rats cannot have resulted exclusively from glomerular injury, and must have been influenced by other factors. One speculative explanation could be that the tubular cells associated with hypotrophic glomeruli might, as postulated for other experimental models, undergo epithelial-mesenchymal transforma-
Fig. 5. A: representative micrographs of renal tissue showing the variability of glomerular areas in C and L\text{Lact} at 3 and 10 mo of age. A population of exceedingly small glomeruli is seen in L\text{Lact}. That these are indeed small glomeruli and not normal glomeruli sectioned close to a pole is shown in the inset, in which the hilum of one of these glomeruli can be easily seen. B: frequency distribution of glomerular areas in C (top) and L\text{Lact} (bottom) at 3 mo (left) and 10 mo (right). Distribution was strongly non-Gaussian in L\text{Lact}, showing a subpopulation of small glomeruli at both 3 (n = 10/group) and 10 (n = 13/group) mo of age. C: bar graph showing the proportion of small, medium-size, and large glomerular areas in C and L\text{Lact}. Both glomerular tuft profiles with small areas (indicating hypotrophy) and with large areas (indicating compensatory hypertrophy) were much more numerous in L\text{Lact} than in C (P < 0.05).
tion and originate myofibroblasts, which are known to actively synthesize components of the interstitial matrix (23).

In addition to the severe structural changes observed in the glomeruli and the interstitium, which led to GFR reduction and creatinine retention, L_{Lact} rats exhibited evidence of tubular dysfunction. At 3 mo of age, when albuminuria, interstitial expansion, and glomerulosclerosis were still mild, urine osmolality was ~25% lower than in C rats, while urine flow was correspondingly increased. This abnormality was aggravated at 10 mo of age, when urine flow more than doubled, and urine osmolality was reduced to <50% compared with age-matched C rats. These findings, which corroborate previous reports (9), suggest that the concentrating ability of L_{Lact} rats is impaired in disproportion to renal structural injury. The reasons for this abnormality are unclear. One possible explanation is that the delicate operation of the countercurrent mechanism is exquisitely dependent on the complex anatomic arrangement of tubular and vascular structures at the renal medulla and might be disrupted by papillary atrophy or even by mild expansion of the renal interstitium (9). Renal concentrating ability could be further compromised by depletion of AQP2, which has been described in rats treated with an ACE inhibitor during lactation (9). Depletion of the Na-K-2Cl cotransporter at the thick ascending limb of Henle has also been described in these rats (16) and might lead to a salt-losing state, with polyuria and diminished concentrating ability.

It is noteworthy that, even in the presence of a chronic, progressive nephropathy, with substantial reduction of the nephron number and of the GFR, systemic hypertension did not develop in L_{Lact} rats until after 6 mo of life, when renal injury had already attained an advanced state. The reasons for this unexpected finding are not immediately apparent. However, it must be noted that, as discussed earlier, tubular function may be impaired in these rats, which may be prone to salt loss (7). Such abnormality would compensate for the inevitable trend toward salt retention imposed by the development of chronic kidney injury and prevent blood pressure elevation until nephron loss and interstitial inflammation were severely aggravated by progression of the disease.

In view of the characteristics and the time course of the ensuing nephropathy, administration of losartan during lactation constitutes a suitable model of chronic progressive nephropathy, is easily reproducible, and requires no surgery or special preparation. An additional advantage of this model is its low mortality, likely associated with the absence of hypertension during its initial stages, allowing the progression of the nephropathy at a relatively slow rate and, consequently, the development of progressive renal insufficiency and of the adaptations and maladaptations that characterize human chronic nephropathies.

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REFERENCES


