Cytoprotective role of heme oxygenase (HO)-1 in human kidney with various renal diseases

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Heme oxygenases (HOs) are rate-limiting enzymes that catalyze the conversion of heme into biliverdin, carbon monoxide and iron [1–3]. Biliverdin is subsequently converted to bilirubin by biliverdin reductase and has potent antioxidant and anticomplement effects [4, 5]. Carbon monoxide acts as a potent vasodilator and inhibitor of platelet aggregation [6, 7]. Furthermore, degradation of heme by HOs leads to the induction of ferritin synthesis, which may then sequester free iron and prevent it from participating in subsequent oxidative injury [8]. Therefore, HOs have antioxidant capacity and act as potent anti-inflammatory proteins whenever oxidative injury takes place. They consist of three different isoforms. One is HO-1, which is an inducible form of the enzymes. The second is HO-2, which is a constitutive form of the enzyme expressed within the brain and testis. Recently, HO-3, which acts like HO-2, has been reported [9].

Our experience of the first human case of HO-1 deficiency reported the precise clinical findings [10] and renal pathology [11]. These findings indicate that tubulointerstitial damage advanced progressively due to the absence of HO-1. However, in HO-1–targeted mice, the glomeruli are the most severely damaged structure, whereas the tubuli remain relatively intact even at 52 weeks, suggesting that HO-1 production is indispensable in protecting glomerular structures in mice. In addition to species differences, the nature of noxious stimuli, modes of administration, and duration of a particular stress may greatly influence the type of renal cell injury and spatial localization of HO-1 production.

\textbf{Key words:} oxidative stress, tubular epithelium, tubulointerstitial injury, renal disease, antioxidant, rate-limiting enzyme.
stress and the subsequent increase in protein carbonyl modification [12–15]. Immunohistochemical studies of CML and pentosidine expression in renal tissues from mouse kidney with unilateral ureteral obstruction and from patients with diabetic nephropathy and IgA nephropathy have been reported previously. In glomeruli of diabetic nephropathy, immunostaining of CML and pentosidine was mainly observed in the expanded mesangial area and capillary walls and strongly in nodular lesions [13, 14]. In contrast, immunoreactivities were only faint in glomeruli of IgA nephropathy [14]. Furthermore, CML proteins were detected within the interstitium of unilateral ureteral obstruction kidneys ten days after the onset of ureteral obstruction [12]. These findings suggest that CML and pentosidine production are closely linked to various degrees of oxidative stress and are greatly enhanced in the case of HO-1 deficiency, due to the lack of a salvage pathway to scavenge severe oxidative stress.

We studied the characteristics of HO-1 production within the kidney in various renal diseases and compared the patterns in relationship to clinical features.

METHODS

Subjects

A total of 74 patients were enrolled in this study. Diagnoses were confirmed by histopathological evaluation of renal biopsy specimens, comprising light microscopy, electron microscopy, and immunofluorescence staining. Included among these were 6 cases of minimal-change nephrotic syndrome (MCNS), 3 cases of focal segmental glomerulosclerosis (FSGS), 50 cases of mesangial proliferative glomerulonephritis (Mes-PGN), 3 cases of IgA nephropathy (IgAN) purpura nephritis and non-IgA nephropathy, 7 cases of tubulointerstitial nephritis (TIN), 3 cases of acute tubular necrosis (ATN), 4 cases of rapidly progressive glomerulonephritis (RPGN), and 1 case of human HO-1 deficiency. In most cases, materials for histopathological examinations were obtained from these patients by renal biopsy. Autopsy samples were obtained from two patients, one of RPGN patients and the HO-1–deficient case. Only three cases showed elevated serum creatinine above the age-matched normal value. All of the patients with MCNS were frequent relapers or steroid-dependent cases, and a renal biopsy was performed to distinguish it from other forms of renal diseases. Renal specimens from the patients with MCNS were obtained at the complete remission stage with or without oral steroid. None of the patients with MCNS were medicated with other immunosuppressive agents. Proteinuria was absent in all of these patients with MCNS at the time of renal biopsy. Duration of these diseases was variable, from several days to more than several years. The characteristics of these cases are shown in Table 1.

### Table 1. Characteristics of the enrolled patients

<table>
<thead>
<tr>
<th></th>
<th>Number of patients</th>
<th>Sex</th>
<th>Mean age elevated serum creatinine N</th>
<th>Patients with elevated serum creatinine N</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCNS</td>
<td>6</td>
<td>6/0</td>
<td>11.8 ± 4.1</td>
<td>0/6</td>
</tr>
<tr>
<td>FSGS</td>
<td>3</td>
<td>2/1</td>
<td>2.0 ± 0.1</td>
<td>0/3</td>
</tr>
<tr>
<td>Mes-PGN</td>
<td>50</td>
<td>27/23</td>
<td>11.8 ± 4.2</td>
<td>0/50</td>
</tr>
<tr>
<td>TIN</td>
<td>7</td>
<td>1/6</td>
<td>11.2 ± 4.4</td>
<td>1/7</td>
</tr>
<tr>
<td>ATN</td>
<td>3</td>
<td>2/1</td>
<td>10.3 ± 1.1</td>
<td>1/3</td>
</tr>
<tr>
<td>RPGN</td>
<td>4</td>
<td>1/3</td>
<td>69.9 ± 1.1</td>
<td>0/4</td>
</tr>
<tr>
<td>HO-1 deficiency</td>
<td>1</td>
<td>1/0</td>
<td>—</td>
<td>0/1</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>40/34</td>
<td>14.8 ± 15.5</td>
<td>3/74</td>
</tr>
</tbody>
</table>

Abbreviations are: MCNS, minimal change nephrotic syndrome; FSGS, focal segmental glomerulosclerosis; Mes-PGN, mesangial proliferative glomerulonephritis; TIN, tubulointerstitial necrosis; ATN, acute tubular necrosis; RPGN, rapidly progressive glomerulonephritis; HO-1, heme oxygenase-1.

Urinalysis

Routine urinalysis and biochemical study were performed immediately and the residual samples were stored at −80°C for future analysis. Hematuria and proteinuria were evaluated by dipsticks. Microscopic hematuria was graded on a scale of (−), (±), (1+), (2+), and (3+). Proteinuria was graded by : (−) absent; (±) ~10 mg/dL; (1+) ~30 mg/dL; (2+) ~100 mg/dL; (3+) ~300 mg/dL; and (4+) ~1000 mg/dL. Urinary N-acetyl-β-D-glucosaminidase (NAG), β₂-microglobulin (β₂m) and creatinine levels were measured at our hospital laboratory.

Processing of renal tissue

Renal tissue samples for light microscopy examination and immunohistochemical studies were fixed in 4% buffered paraformaldehyde, dehydrated by graded concentrations of ethanol and xylene, and then were embedded in paraffin. For the routine immunofluorescence studies, 4 μm thick frozen sections were fixed in cold acetone and stored at −20°C until examined. The specimens were investigated for the deposits of immunoglobulin (Ig) G, IgA, IgM, complement (C)1q, C3, C4, and fibrinogen using the direct immunofluorescence method. For routine electron microscopic examination, the samples were fixed in glutaraldehyde (2% wt/vol in 0.1 mol/L cacodyl...
buffer, pH 7.4) for two hours at 4°C. The samples were washed with the buffer and postfixed in 1% osmium tetroxide in 0.1 mol/L cacodyl buffer for one hour. Then these samples were dehydrated and embedded in epon resin. The sections (100 nm) were stained with uranyl acetate for 15 minutes, followed by saturated lead citrate for seven minutes before being examined under a transmission electron microscope.

**Immunohistochemistry**

Immunostaining for HO-1 or macrophage-specific antigen, CD68 (KP-1), was performed with an anti-HO-1 rabbit antiserum (StressGen, Victoria, British Columbia, Canada) or an anti-CD68 monoclonal antibody (Dako Co., Carpinteria, CA, USA). Briefly, paraffin sections of the kidney were deparaffinized and rehydrated with descending ethanols. For HO-1 staining, the sections were immersed in Target-Retrieval Solution (Dako) and heat-treated in a microwave oven for five minutes at high power (500 W). Then the slides were immersed in hot Target-Retrieval Solution to cool for 30 minutes and rinsed in Tris buffer. For CD68 immunostaining, the slides were incubated with pronase (0.5 mg/mL; Dako) for 15 minutes at room temperature in a buffer solution containing 0.05 mol/L Tris-HCl (pH 7.2) and 0.1 mol/L NaCl. The slides were heat-treated in the same fashion as for the HO-1 staining. After blocking with normal goat serum, the sections were incubated with appropriate dilutions of each first antibody for 12 hours at 4°C. After washing the slides in Tris buffer, tissue sections were incubated with EnVision polymer reagents (Dako), alkaline phosphatase and goat anti-rabbit/mouse IgG conjugated dextran polymer, for 30 minutes at room temperature. Alkaline phosphatase activity was visualized using Fast Red TR salt (Sigma Chemical Co., St. Louis, MO, USA) after further washing in Tris buffer.

Immunostaining for CML or pentosidine was performed with an anti-CML or anti-pentosidine rabbit antiserum, respectively [12–14]. Paraffin sections of the kidney were deparaffinized and rehydrated with descending ethanols. They were further incubated with pronase (0.5 mg/mL; Dako) for 15 minutes at room temperature in a buffer solution containing 0.05 mol/L Tris-HCl (pH 7.2) and 0.1 mol/L NaCl. The sections were washed with phosphate-buffered saline (PBS) containing 0.05% Tween and blocked with normal goat serum for 20 minutes, and then the sections were incubated with anti-CML rabbit antiserum (10 μg/mL) or anti-pentosidine rabbit antiserum (20 μg/mL) for 12 hours at 4°C. After washing the slides in Tris buffer, tissue sections were incubated with goat anti-rabbit IgG conjugated with peroxidase (Dako) for two hours at room temperature. Peroxidase activity was visualized using a diaminobenzidine (DAB) solution.

Immunostaining for Tamm-Horsfall protein (THP) was performed with an anti-THP sheep serum and alkaline phosphatase-conjugated donkey anti-sheep second antibody (Chemicon Int. Inc., Temecula, CA, USA). The slides were deparaffinized and heat-treated as described previously in this article. Isotype-matched mouse IgG, normal rabbit serum or normal sheep serum was used as negative controls for the immunostainings.

**Evaluation of HO-1, CML, and pentosidine staining**

Intensity of the immunostainings within the glomeruli, proximal tubuli, and the distal tubuli was evaluated separately. Proximal tubuli were differentiated from distal tubuli as follows: Proximal tubuli had larger diameters than distal tubuli. The nuclei of proximal tubular epithelial cells generally were arrayed at the base of the cells. Most proximal tubular epithelial cells had obvious brush borders. Furthermore, in some cases with severe tubulo-interstitial damage, the staining of THP was examined, which was a specific marker for distal tubuli. Two consecutive specimens for HO-1 and THP stainings were compared to differentiate proximal tubuli from distal tubuli. Based on the intensity and distribution of HO-1 staining, the degrees of HO-1 staining were classified into point 0 to point 4 as follows: point 0, no staining; point 1, weak and focal; point 2, weak and diffuse or moderate and focal; point 3, moderate and diffuse or strong and focal; point 4, strong and diffuse (Fig. 1). Five nephrologists examined the specimens independently and judged the staining patterns without knowledge of the patient’s histological diagnosis. Mean points ± SD were calculated. CML and pentosidine staining patterns were evaluated by the same method as HO-1.

**Statistics**

Data are expressed as mean ± SD. For comparison of two groups, any one of the statistical tests, the Mann-Whitney test or Fisher exact probability test, was used as appropriate. Values are considered statistically significant at \( P < 0.05 \).

**RESULTS**

**Spatial localization of renal HO-1 production**

Heme oxygenase-1 was not detectable in any part of the kidney specimen from the HO-1–deficient patient. In marked contrast, expression of HO-1 protein was recognized within the renal tubular epithelial cell in virtually all cases examined. HO-1 protein was not detected within glomerular intrinsic cells, including mesangial cells, glomerular endothelial cells, and glomerular epithelial cells. There were a few HO-1–positive cells scattered within the glomeruli and the interstitium. These cells exhibited characteristic morphology with abundant cytoplasm and indented nuclei (Fig. 2 A, B). Immunohistochemical examination of the consecutive sections revealed that HO-1 producing cells were CD68-positive
Fig. 1. Evaluation of heme oxygenase-1 (HO-1), \(N\)-carboxymethyllysine (CML), and pentosidine staining. Intensity of HO-1 staining within glomeruli, proximal tubuli and distal tubuli was evaluated by immunohistochemistry. Representative figures for negative, weak, moderate, and strong staining are shown.

Fig. 2. Immunohistochemical demonstration of HO-1– and CD68-positive macrophages. HO-1 staining cells were detected within the interstitium (A, original magnification \(\times400\)). A high-power view of the HO-1 staining cell shows its abundant cytoplasm and characteristic indentation of the nucleus (B, original magnification \(\times1000\)). Immunohistochemical examination of consecutive renal sections indicated that HO-1 staining cells within the Bowman’s space and within the interstitium (C and E, original magnification \(\times200\)) were both CD68-positive macrophages (D and F, consecutively, original magnification \(\times200\)).

(Fig. 2 C–F). The results strongly indicated that these HO-1–producing cells were rare macrophages, rather than intrinsic glomerular cells. None of the blood vessel components within the kidney expressed HO-1 protein in any of the diseases. HO-1 staining within distal tubuli tended to be more intense (2.56 ± 0.70 points) than proximal tubuli (1.95 ± 0.68 points) in all of the specimens (Mann-Whitney test, \(P < 0.05\)).

**HO-1 expression in various renal diseases**

Figure 3 shows the expression of HO-1 in various renal diseases including the HO-1–deficient case (Fig. 3A), advanced FSGS (Fig. 3B), RPGN (Fig. 3C), ATN (Fig. 3D), IgAN (Fig. 3E) and MCNS in complete remission (Fig. 3F). It was intriguing that characteristic HO-1 staining also was observed within tubular cells of patients with minimal change nephrotic syndrome in complete remission, in which no apparent tubular damage usually is seen. There was no statistical significance in the intensity of HO-1 staining within proximal tubuli between MCNS and Mes-PGN. Compared with MCNS or Mes-PGN, the intensity of HO-1 staining was significantly stronger within proximal tubuli in FSGS and RPGN (Mann-Whitney test, \(P < 0.05\); Fig. 4A). In contrast, the intensity of HO-1 staining within proximal tubuli was not stronger in ATN and TIN as compared with MCNS or Mes-PGN. Within distal tubuli, the intensity of HO-1 staining did not differ significantly among all of the diseases (Fig. 4B).

**Correlation between intensity of HO-1 production and degree of hematuria, proteinuria or other clinical characteristics**

In Mes-PGN, HO-1 staining within proximal tubuli tended to be more intense with greater degrees of hematuria (Mann-Whitney test, \(P < 0.01\); Fig. 5A). Although there is no direct correlation between the intensity of HO-1 staining within proximal tubuli and the grade of proteinuria, patients with proteinuria showed more intense expression of HO-1 (≥2.5 points) within proximal tubuli than the patients without proteinuria (by the Fisher’s exact probability test, \(P < 0.01\); Fig. 5C). There was no correlation between the intensity of HO-1 staining and degrees of hematuria or proteinuria within distal tubuli (Fig. 5 B, D). Age and sex, blood pressure, degree of serum creatinine levels, urinary NAG levels and urinary \(\beta\)-m levels of the patients did not seem to affect HO-1
HO-1 was not detected in the renal specimen of the HO-1–deficient case (A), but was detected in all of the various renal diseases, namely, advanced focal segmental glomerulosclerosis (B), rapidly progressive glomerulonephritis (C), acute tubular necrosis (D), IgA nephropathy (E), and minimal change nephrotic syndrome in remission (F).

Expression of CML and pentosidine in renal diseases

Nε-carboxymethyllysine and pentosidine were detectable within glomeruli and tubuli in renal tissues from patients with various renal diseases, but only at low levels. These staining patterns showed no particular tendency and did not correlate with other clinical data, such as degree of proteinuria, hematuria, urinary NAG levels, or urinary β₂m levels (data not shown). It was striking that intense immunostainings of CML and pentosidine were observed within both proximal and distal tubuli in the biopsy and/or autopsy renal specimens of HO-1–deficient patient. In contrast, they were only faintly detectable within epithelial cells of the proximal tubuli and glomeruli in renal tissues from patients with IgA nephropathy (C and D).

DISCUSSION

Heme oxygenase-1 is an inducible enzyme, and its induction is important in the response of tissues to oxidative stress and inflammation. Recent studies have focused on the biologic effects of the reaction products that potentially possess important antioxidant, anti-inflammatory, antiapoptotic, and immune modulatory functions [3, 16–25]. Nath et al elegantly proposed one such protective
Fig. 4. Quantitative analysis of HO-1 staining in various renal diseases. The intensity of HO-1 staining was significantly stronger within the proximal tubuli in renal tissues from advanced FSGS and RPGN patients who had moderate tubulointerstitial damage than those from MCNS or Mes-PGN patients (Mann-Whitney test, \( P < 0.05 \); A). However, the intensity of HO-1 staining within renal proximal tubuli of ATN or TIN, which had the most severe tubulointerstitial damage, was not significantly strong. There was no significant difference in the intensity of HO-1 staining within the distal tubuli among various diseases (B).

Fig. 5. Correlation between the intensity of HO-1 staining and the level of hematuria or proteinuria. In mesangial proliferative glomerulonephritis (mes-PGN), HO-1 staining within proximal tubuli tended to be more intense with greater degrees of hematuria (Mann-Whitney test, \( P < 0.01 \); A). Although there was no direct correlation between the intensity of HO-1 staining within proximal tubuli and grade of proteinuria, patients with proteinuria showed more intense expression of HO-1 (\( \geq 2.5 \) points) within proximal tubuli than the patients without proteinuria (Fisher’s exact probability test, \( P < 0.01 \); C). Within distal tubuli, there was no correlation between the intensity of HO-1 staining and degree of hematuria or proteinuria (B and D). In (C), open circles indicate intense expression of HO-1 (\( \geq 2.5 \) points), and closed circles indicate weak expression of HO-1 (<2.5 points).

Mechanism in a rat model of glycerol-induced acute renal failure [26]. In their model, rapid induction of HO-1 mRNA and protein was observed within the kidney by a single prior injection of hemoglobin, which drastically prevented progression of renal failure. However, the extent of the glomerular damage and the exact location of HO-1 production within the kidney were not specified in their report. Furthermore, renal function was protected when other inducers of HO-1, such as endotoxin, hemin, and cisplatin, were administrated prior to the induction.
of acute renal failure in the rat models [27, 28]. Other reports also have shown that HO-1 has important protective roles in preventing oxidative injury in the renal tubuli in various animal models [29–33]. The majority of these reports suggest that HO-1 production is localized to the tubular epithelial cells in animal models. However, several reports have indicated that mesangial cells produce HO-1 in vitro culture systems [34, 35]. Mosley et al demonstrated for the first time that HO-1 was induced within the glomeruli of proliferative glomerulonephritis and identified the major HO-1–producing cells as macrophages by immunohistochemistry [27]. Our present study found that HO-1 production was confined within tubular epithelial cells, regardless of the nature of renal diseases. HO-1–producing cells were occasionally recognized within the glomeruli and interstitium, but these were identified as CD68-positive macrophages. These results suggest that tubular epithelial cells are the principal HO-1 producer in vivo. Further study is necessary to elucidate whether other cell types produce HO-1 in specific disease conditions.

We previously described the renal histopathological changes present in the first reported (to our knowledge) human case with HO-1 deficiency [11]. Tubulointerstitial injury with subsequent tubular dilation and/or atrophy, inflammatory cell infiltration and interstitial fibrosis seemed to be the major pathological findings in this patient. In an earlier study, Poss et al reported the renal pathological changes in HO-1 targeted mice. In contrast to the human HO-1–deficient case, the glomeruli seemed to be more vulnerable than the tubular structures in the HO-1 targeted mice, with widespread injury noted in the glomeruli [23]. It is not clear why there are such marked differences in the pathological findings between human HO-1 deficiency and HO-1 targeted mice. There seem to be fundamental differences in both the environment and the total duration of stress exposures. HO-1 targeted mice were presumably bred in a sterile, pathogen-free environment, without the danger of ubiquitous infectious agents, and the mice survived for a long period of time. The renal lesions are, therefore, the result of a long-standing but low level of stress to the kidney. However, our patient was exposed to unremitting waves of infectious agents, starting shortly after birth. Our hypothesis has been supported by two reports describing the renal injury in the HO-1 knockout mouse. Nath et al observed a significant reduction of renal function with severe tubular injury accompanied by 100% mortality in the HO-1 knock−/− mice when they were exposed to glycerol [36]. In sharp contrast, renal dysfunction was only mild and reversible and was not accompanied by mortality in HO-1 +/+ mice. Hemoglobin also induced rapid renal damage in both young and old HO-1–deficient mice, whereas it had little effect on normal mice. Using immunohistochemistry, they found that the principal site of HO-1 production after glycerol challenge was the proximal tubuli, but not the glomeruli. Our HO-1−deficient case suffered from systemic vascular endothelial cell injury, with massive intravascular hemolysis. The serum was loaded with heme and a large amount of heme-conjugated haptoglobin, and a high concentration of haptoglobin was detectable in the urine. As a result, tubular epithelial cells became the sites of active haptoglobin uptake and hemoglobin metabolism, leading to accumulation of haptoglobin within these cells. Consequently, the renal tubular epithelium in this patient has been constantly exposed to high concentration of heme with severe tubular damage [11]. Another report by Shiraishi et al described that HO-1–targeted mice develop more severe renal damage and have significant renal tubular injury (apoptosis and necrosis) compared with wild-type mice when they were treated with cisplatin. These authors again showed that significant HO-1 production in vitro was inducible in proximal tubular cells [37]. Thus, these results strongly indicate that renal tubular epithelial cells are constantly exposed to various oxidative stresses in vivo, and therefore are vulnerable to injury whenever the level of the insults becomes overwhelming. Collectively, selective HO-1 production within these cells will play an important protective role in preserving renal functions.

Next, we examined the influence of various factors that are responsible for the induction of HO-1 production. Because the number of available cases was limited, cases were selected with Mes-PGN for the comparative study to elucidate the roles of hematuria or proteinuria in the induction of HO-1 within renal tubuli. In these Mes-PGN patients, HO-1 staining within proximal tubuli tended to be more intense with greater degrees of hematuria. The result suggests that hematuria is one major such oxidative stress to induce HO-1 production in the case of glomerular damage. Recently, Nath et al reported the same results in the patients with paroxysmal nocturnal hemoglobinuria [38]. On the other hand, there was no direct correlation between the intensity of HO-1 staining within proximal tubuli and grade of proteinuria, although there was a significant difference in HO-1 production between patients with proteinuria and without proteinuria. In addition to heme, glomerular proteinuria contains various materials that may also become an oxidative stress to tubular epithelial cells by directly contacting with the cells at high concentrations. Proteinuria is the net result of both glomerular and tubular damage. Accordingly, the degree of the proteinuria per se may not directly correlate with the degree of tubular damage. However, proteinuria may result from tubular injury induced by inflammation and other oxidative stresses. In fact, the fraction of patients with intense HO-1 staining in the proximal tubuli was significantly larger among those with greater degrees of proteinuria, indicating that
at least some of the cases with massive proteinuria are caused by severe tubular injury associated with HO-1 up-regulation. Furthermore, in the cases without apparent hematuria and proteinuria, glomerular filtrate may contain various materials that become oxidative stresses. Constant HO-1 production thus may serve as an important protective mechanism for the epithelial cells even in normal kidney. Therefore, HO-1 expression seen in the MCNS kidney may reflect the baseline physiological level of the enzyme expression. Kupffer cells of the liver and splenic macrophages also are known to constantly produce HO-1, reflecting their physiological roles in each organ [2, 10].

The reason that no significant level of HO-1 expression was seen in ATN or TIN is not clear. However, it is possible that the degree of HO-1 expression correlates more with the level of oxidative stress to the tubular epithelium at the time of biopsy, rather than the cumulative tissue injury caused by an insult. Furthermore, we did not see any significant correlation between HO-1 expression and urinary NAG or β2m concentrations, further supporting the view that HO-1 expression reflects the ongoing cellular stress, but not its consequences, such as cellular injury or functional disturbance of the tissue. In addition, the insult to the cells may result in rapid apoptosis or cell death without an appreciable induction of HO-1, when the degree of the oxidative stress is overwhelming and acute. Relatively low levels of HO-1 expression and the presence of apoptotic cells within the tissue in ATN and TIN indicate that severe and acute cell injury was induced in these conditions, rather than persistent and moderate oxidative stress. Mouse models of tubular injury induced with or without prior sensitization may support these in vivo data [27, 28].

It is not clear why there are significant differences in the expression patterns of HO-1 between proximal and distal tubular epithelial cells and why the moderate expression of HO-1 protein is constantly recognized within distal tubular epithelial cell in virtually all of the cases examined. Recent studies have reported low levels of constitutive expression of HO-1 in the renal inner medulla, where it may contribute to the maintenance of the renal medullary circulation [39]. It is possible that the constantly moderate level of HO-1 expression within distal tubuli seen in various renal diseases indicates that HO-1 expression in vivo contributes to the maintenance of the renal circulation, in addition to the protection of tubular cells from oxidative injury. Furthermore, Liang et al’s preliminary report showed the differential induction of HO-1 in a renal proximal tubular cell line and renal distal tubular cell line. They concluded that the renal expression of HO-1 was regulated in a tubule segment-specific manner and/or species-specific fashion, and that such regional heterogeneity in the expression of HO-1 may be relevant to the different functions of HO-1 played in renal injury (abstract; Liang et al, J Am Soc Nephrol 11:604A, 2000).

Our study evaluated CML and pentosidine in the kidney as surrogate markers of oxidative stress. Both CML and pentosidine are formed mainly under oxidative stress by carbonyl amine chemistry between protein amino groups and carbonyl compounds, and thus have been suggested as integrative biomarkers of cumulative oxidative stress in various tissues including kidney [15]. Several studies have shown that CML and pentosidine are accumulated within the kidney [12–14]. They have been identified within the expanded mesangial matrix and nodular lesions in diabetic nephropathy and in the interstitium of unilateral ureteral obstruction, which is a well-established experimental model of renal injury leading to interstitial fibrosis. In IgA nephropathy, their immunoreactivities are only faint in the glomeruli. In renal tissues from patients with various renal diseases, immunostaining of CML and pentosidine was detected, but was only faint in both glomeruli and tubuli. In contrast, intense immunostaining was specifically observed within the renal tubular epithelial cells in renal tissue from the HO-1–deficient case. These results clearly indicate that the tubular epithelial cells in HO-1 deficiency had been exposed to persistent, severe oxidative stress, which was markedly potentiated by the absence of HO-1. In contrast, progressive accumulation of oxidative stress is attenuated in HO-1–positive individuals by the potent cytoprotective effect of HO-1, which is rapidly induced within tubular cells in response to environmental insults.

In conclusion, these results show that HO-1 plays pivotal roles in the maintenance of renal functions and protection of renal structures under oxidative stress, especially within the renal tubular epithelial cells. Further studies are necessary to elucidate the spatial localizations and patterns of HO-1 expression and to understand the regulatory mechanism of HO-1 production within the kidney. The mechanism of antioxidative injury in the kidney will be the focus of future studies, which will further clarify the role of HO-1 in the pathogenesis of various renal diseases.

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