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HO-1 expression control in the rat glomerulus

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ABSTRACT

The differential localization of HO-1 in renal cells under conditions of injury, and the demonstration that exaggerated HO-1 expression can have detrimental rather than beneficial effects, raises the question of whether HO-1 expression in these cells is subject to control. The present study identifies a unique HO-1 expression pattern in the renal glomerulus indicative of presence of HO-1 expression control following prolonged HO-1 induction. HO-1 and HO-2 expression in response to the natural HO substrate/inducer Fe^{++} protoporphyrin (PP) IX (hemin) was assessed in normal rat glomeruli. Following 18 h incubations with hemin (0–200 μM), HO-1 expression increased in a concentration-dependent manner and via a hemopexin (HPX) independent mechanism with no effect on HO-2. In incubations with higher hemin concentrations (400 μM), likely to be encountered in hemolytic disorders, HO-1 expression, decreased. This was preceded by a prolonged and sustained increase in HO-1 protein and was independent of the Fe^{++} moiety as incubations with Cobalt protoporphyrin (CoPP) resulted in an identical expression pattern. The decrease of HO-1 protein could not be accounted for by proteasomal degradation since it was not reversed in co-incubations with hemin and the proteasome inhibitor, MG132, at concentrations sufficient to increase HO-1 glomerular content when used alone. Moreover, in the presence of MG132, a decrease of HO-1 expression also occurred at 100 and 200 μM hemin. The effect of MG132 was mimicked by two additional mechanistically different approaches which also raised HO-1 content: a) co-incubations of hemin with ZnPP which increased HO-1 protein when used alone, and b) glomerular HO-1 over expression achieved by SB transposon mediated transgenesis. In contrast, the decrease in HO-1 levels observed at high hemin concentrations was reversed in co-incubations with hemin and SnPP, which reduced HO-1 content when used alone. Expression of NF-E2 related factor 2 (Nrf2) protein, which mediates HO-1 induction in response to hemin, had a similar expression pattern with that of HO-1 protein indicating involvement of Nrf2 in the response of HO-1 to hemin. The above observations indicate presence of a HO-1 expression control mechanism in the glomerulus that may serve to protect it against potentially detrimental effects of exaggerated HO-1 expression.

1. Introduction

The cytoprotective effect of heme oxygenase (HO) is well established. The inducible isoenzyme, HO-1, is expressed in most tissues at low levels, but can respond to stress with rapid transcriptional activation of the HO-1 gene. It catalyzes degradation of heme, it's natural substrate, to biliverdin and carbon monoxide (CO) with simultaneous release of catalytically active (reactive) Fe^{++}. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. With the exception of Fe^{++}, all heme degradation products were shown to have cytoprotective effects [1]. However, exaggerated HO-1 induction can occur in certain clinically relevant disease models [2] and may not be beneficial as the release of reactive iron may mediate reactions leading to hydroxyl radicals formation [3], and also reverse HO-1 related cytoprotection [4].

A marked and sustained HO-1 expression in tissues can be encountered in disorders of systemic hemolysis, in which concentrations of free heme in the circulation can approach 200 μM

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[5,6]. Among these tissues are microvascular beds including the glomerular microvasculature of the kidney (glomeruli), in which, free heme is filtered. In glomeruli, increased exposure to heme may also occur in various forms of glomerular injury associated with hematuria, as in aggressive variants of glomerulonephritis, in which haemoglobin (Hb) is released from red blood cells (RBC) undergoing membrane damage while passing through glomeruli [7]. In these forms of injury a strong pro-oxidant environment also develops due to overproduction of reactive oxygen and nitrogen radicals [8]. The latter promote oxidation of RBC-derived Hb to methemoglobin and release of ferric heme [9], which is expected to markedly increase in HO-1 expression in glomerular cells.

However, a number of studies have shown limited or complete absence of HO-1 expression both in haemolytic disorders and in clinical and experimental forms of glomerular injury. Instead, a robust increase of HO-1 expression was shown in tubular epithelial cells and infiltrating inflammatory leukocytes. This was convincingly shown in antibody-mediated haemolytic anemia [10] and in a spectrum of glomerular diseases, including rapidly progressive glomerulonephritis, mesangioproliferative glomerulonephritis, focal segmental glomerulosclerosis and minimal change nephropathy [11]. In these diseases, HO-1 mRNA but not HO-1 protein was detectable within glomerular epithelial cells while neither HO-1, mRNA or protein, could be detected in mesangial or endothelial cells [12]. Similar findings were confirmed in experimental models of glomerular injury in which potent HO-1 inducers such as cytokines and pro-oxidant radicals including superoxide ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) are overproduced in the glomerular milieu. A robust HO-1 protein expression was consistently found in infiltrating inflammatory cells and tubular epithelial cells but not in intrinsic glomerular cells [13].

The limited HO-1 expression observed in glomerular cells under conditions of injury *in vivo* contrasts with the demonstration that all glomerular cell types (endothelial, mesangial, epithelial) [14–16], can promptly upregulate HO-1 expression (mRNA and protein) in response to established inducers *in-vitro*. This discrepancy points out a distinct HO-1 expression pattern in the glomerulus as an integrated tricellular structure and, raises the question of whether a mechanism limiting HO-1 expression exists in the glomerulus. The present study addressed this question in isolated rat glomeruli using heme (hemin) as a HO-1 inducer.

2. Materials and methods

2.1. Reagents

Anti-HO-1 and anti-Nrf2 antibodies were purchased from Assay Design and R&D systems respectively. Anti-β-actin, heme (hemin), cobalt protoporphyrin (CoPP), zinc protoporphyrin (ZnPP), and MG-132 were obtained from Sigma-Aldrich, Tin protoporphyrin (SnPP) from Tocris Bioscience. Hemopexin deficient (HPX^-) serum was a kind gift from Dr. Emanuala Tolosano, Molecular Biotechnology Center, University of Torino, Italy.

2.2. Rats

Adult male Spraque-Dawley rats, 300 g in body weight, were employed in this study. Animals were reared in accordance to the European Union Directive for care and use of laboratory animals and all procedures were approved by the Hellenic Veterinary Administration and the ethical committee of 'Evangelismos' Hospital.

2.3. Generation of glomerular epithelial cell (GEC)-targeted HO-1 overexpressing transgenic (TG) rats by Sleeping Beauty (SB) transposon mediated transgenesis

An SB transposon vector (SB-hHO1) was constructed harboring a FLAG-tagged human (h) HO-1 sequence under control of a murine nephrin promoter we previously used to achieve GEC-targeted hHO-1 expression in the mouse [17]. The SB transposon system methodology, as reported by Katter et al. [18], was applied. Briefly, the vector was mixed with SB100 transposase mRNA and injected into the pronucleus of newly fertilized Sprague-Dawley (SD) embryos before transferring to pseudopregnant females. Insertions were mapped by rat genome digestion with Bfa1 and linker-mediated PCR in order to obtain a sequence tag that could be "blasted" thereby determining: a) the specific chromosome location of the transposon and b) whether the transposon was incorporated in an exon or intron. Sequences obtained from LM-PCR were subjected to rat genome analysis by comparison to the Baylor 3.4/rn4 reference sequence at http://genome.ucsc.edu/ to map the precise transgene integration sites.

2.4. Isolation and treatment of glomeruli

Glomeruli were isolated from kidneys of wild type (WT) or TG rats by an established differential sieving method [19] and incubated at 37 °C in a 5% CO_2 environment in Dulbecco's modified Eagle's medium (DMEM) containing 10% complete serum or HPX^- serum. Glomeruli were incubated with defined concentrations of the following metalloporphyrins (MPs): hemin, CoPP, ZnPP and SnPP which were dissolved in dimethyl sulfoxide (DMSO) or co-incubated with hemin and either ZnPP or SnPP, or the proteasome inhibitor, MG132. Negative control samples consisted of glomeruli incubated with vehicle (DMSO) only. Protein extracts were prepared using lysis buffer (150 mM NaCl, 50 mM Tris pH 8.0, and 1% Triton X containing a protease inhibitors cocktail) and concentration was determined by the Bradford assay. RNA was extracted by an established Trizol-based method.

2.5. Western blotting

Protein lysates were resolved by sodium dodecyl sulphate-polyacrylamide electrophoresis (SDS-PAGE), transferred onto polyvinyledinedifluoride (PVDF) membrane and probed with primary antibodies overnight. Horseradish peroxidase conjugated secondary antibodies were used for detection and a chemiluminescence substrate was used for visualisation.

2.6. Reverse transcription reaction and Real-time PCR amplification

Glomerular RNA concentration was determined by spectrophotometry. Reverse transcription reactions were performed using TaqMan Reverse Transcription Reagents kit (Applied Biosystems). Real-time PCR was carried out at the following conditions: 25 °C for 10 min, 48 °C for 30 min and 95 °C for 5 min. Each reaction consisted of 2 μl primer-probe assay mix (IDT), 10 μl Master Mix (Applied Biosystems) and 8 μl cDNA. Values were analysed by the ΔΔCT method.

2.7. Statistical analyses

Values are presented as mean ± SE (standard error). Statistical analyses were performed with either t-test, where applicable, or analysis of variance (ANOVA) for more than two group comparisons. When significant, post hoc analysis was performed, with the

least significant difference (LSD) test. A p value <0.05 was chosen as statistically significant.

3. Results

3.1. Effect of hemin on glomerular HO-1 expression

Incubations with hemin were performed for 18 h at concentrations (6–400 μM) shown to protect against superoxide-mediated cell toxicity [20] or likely to be found in haemolytic disorders (≥200 μM) [21]. Hemin induced glomerular HO-1 expression at concentrations as low as 6 μM (Fig. 1A(i)). The effect of high hemin concentrations (50–400 μM) on HO-1 expression in 18 h incubations is shown in Fig. 1A(ii). Hemin concentrations 50–200 μM increased HO-1 protein to similar levels. This effect was independent of HPX as it also occurred in serum free-media as well as in HPX⁻ deficient serum containing media (Fig. 1B). At higher hemin concentrations (400 μM), 18 h HO-1 protein levels, decreased to near basal (Fig. 1A(ii)). Changes in HO-1 mRNA levels were similar to those in HO-1 protein (data not shown). Changes in levels of NF-E2 related factor 2 (Nrf2) protein, which is known to mediate HO-1 induction in response to heme [22] were also similar to the HO-1 protein expression pattern with an increase following incubations with 200 μM hemin and a reduction to basal in incubations with 400 μM hemin (Fig. 1C). Hemin had no effect on glomerular HO-2 expression (Fig. 1D).

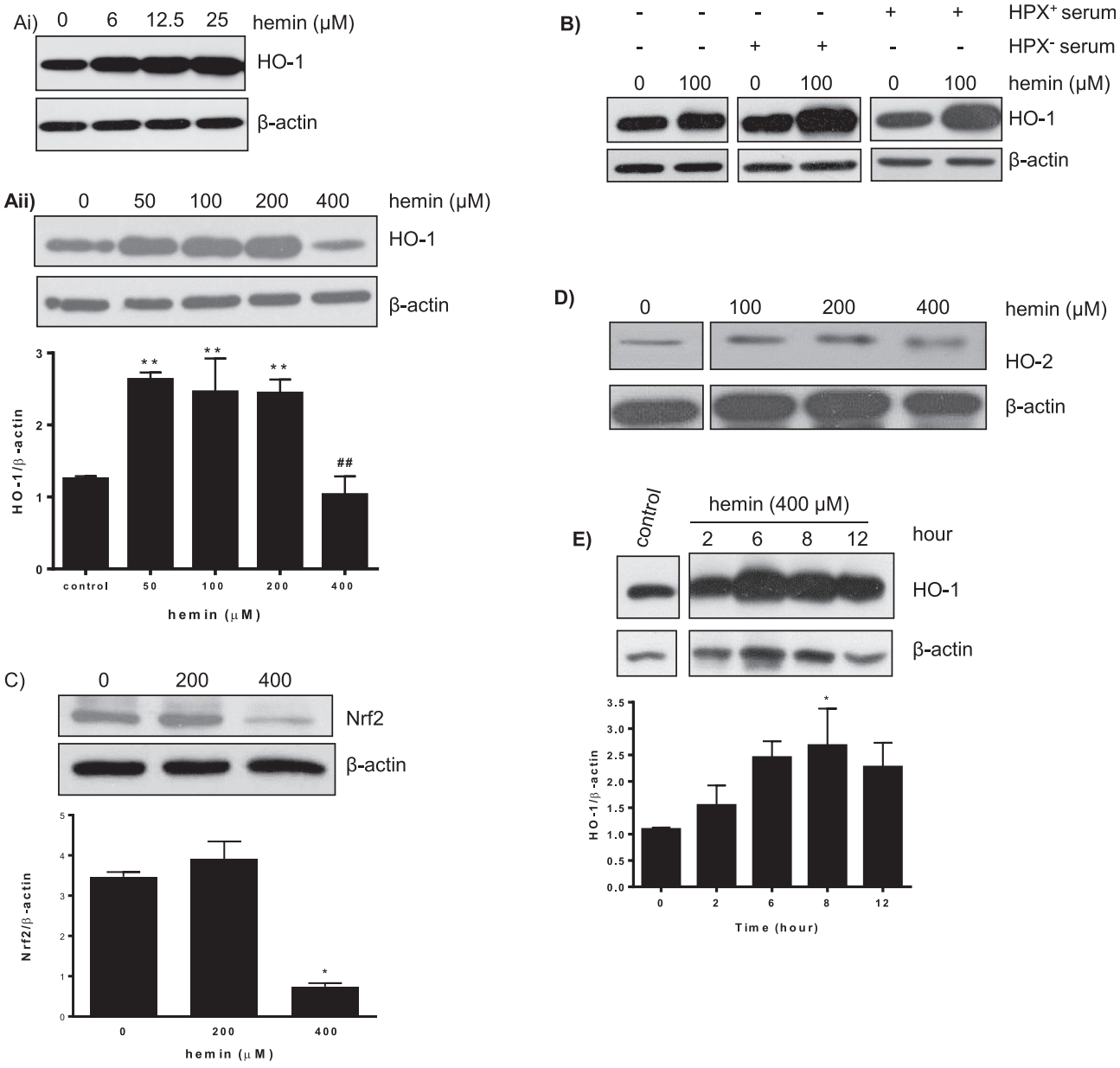

Fig. 1. Hemin-mediated glomerular HO-1 induction: concentration- and time-dependent effect and role of Hemopexin. (A) Glomeruli were incubated with (i) low and (ii) high concentrations of hemin for 18 h. Total protein lysates were immunoblotted for HO-1. β-actin was used as a loading control. Representative western blot and mean ± SEM of densitometry values from three independent experiments are presented in the graph. **p < 0.01 vs no hemin, ##, p < 0.01 vs 200 μM hemin. (B) Glomeruli were incubated with hemin (100 μM) for 18 h in the absence of serum, in the presence of HPX-deficient (HPX⁻) serum or in the presence of HPX-containing serum (HPX⁺) and total protein lysates were immunoblotted for HO-1. (C) Glomeruli were incubated with increasing concentrations of hemin for 18 h and total protein lysates were immunoblotted for Nrf2 or (D) HO-2. (E) Glomeruli were incubated with hemin (400 μM) for various time periods. Total protein lysates were immunoblotted for HO-1. β-actin was used as a loading control. Representative western blot and mean ± SEM of densitometry values from three independent experiments are presented in the graph. *p < 0.05 vs 0 h.

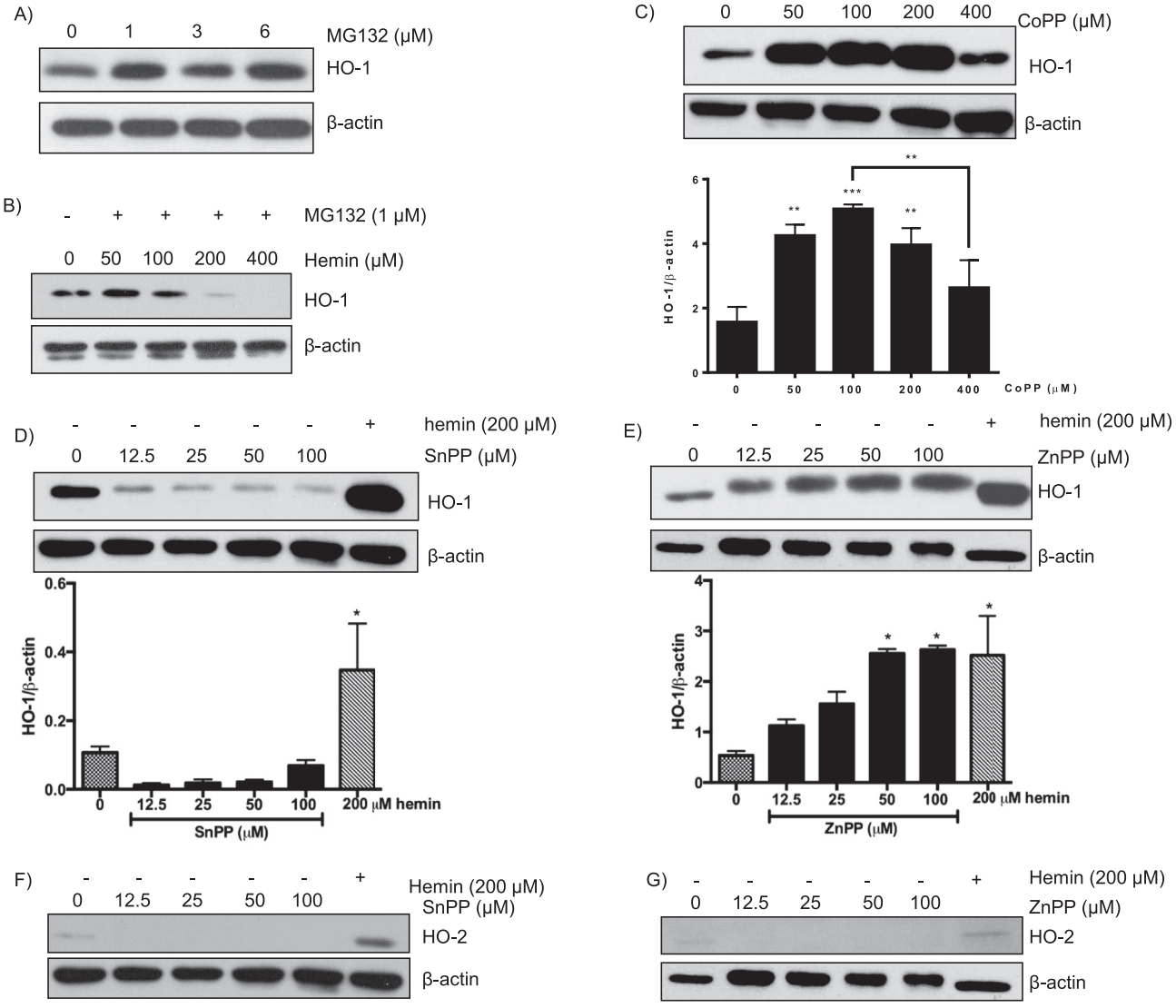

Fig. 2. Effect of MG132 and hemin co-incubations on HO-1 and differential effect of CoPP, SnPP and ZnPP on glomerular HO-1 and HO-2 protein. (A) Glomeruli were incubated with increasing concentrations of the proteasome inhibitor MG132 alone or (B) with increasing concentrations of hemin in the presence of MG132 (1 μM) for 18 h and total protein lysates were immunoblotted for HO-1. Glomeruli were incubated with increasing concentrations of CoPP or SnPP or ZnPP for 18 h and total protein lysates were immunobloted for either HO-1 (C, D and E respectively) or HO-2 (F and G). β-actin was used as a loading control. Representative western blot and mean ± SEM from three independent experiments are presented in the graphs. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs 0 μM MP (SnPP or ZnPP).

The reduction in HO-1 protein observed following 18 h incubations with 400 μM hemin (Fig. 1A(ii)) was preceded by a significant increase in shorter incubation periods (6, 8 and 12 h) (Fig. 1E). Specifically, HO-1 expression increased as early as 2 h and reached a plateau at 8–12 h, indicating that the reduction at 18 h occurred after 12 h of sustained increase in HO-1 synthesis. This raised the question of whether HO-1 protein degradation could have accounted for the reduction in HO-1 protein levels observed at 18 h. To address this question, glomeruli were incubated with hemin in the presence of the proteasome inhibitor MG132 employed at concentrations (1 μM) sufficient to increase glomerular HO-1 protein when used alone (Fig. 2A). As shown in Fig. 2B, HO-1 protein did not increase in co-incubations with 400 μM hemin and MG132. On the contrary, it became undetectable. Moreover, in the presence of MG132, there was no increase in HO-1 protein in response to 100 μM hemin while, in response to 200 μM, HO-1 protein decreased to levels below basal. Collectively, these observations point out that the decrease in HO-1 levels could not be accounted for by proteasomal degradation and suggest that the elevated HO-1 content attained due to inhibition of proteasomal HO-1 degradation negatively regulated HO-1 response to exogenous heme and that this was triggered when HO-1 levels reached a particular threshold. To further characterize this effect, we varied glomerular HO-1 content using non-iron protoporhyrins with opposite effects on HO-1 synthesis as well as glomeruli over expressing HO-1.

3.2. Effect of non-Fe MPs on glomerular HO-1 expression and modulation of response to hemin

These experiments employed three MPs (CoPP, SnPP and ZnPP) differing in the metal moiety chelated with the same macrocycle protoporphyrin (PP) IX, which comprises the ring structure of the natural MP, heme (Fe^{++} PP IX). As shown in Fig. 2C, a HO-1 expression pattern identical to that in response to hemin was observed in 18 h incubations of isolated glomeruli with CoPP.

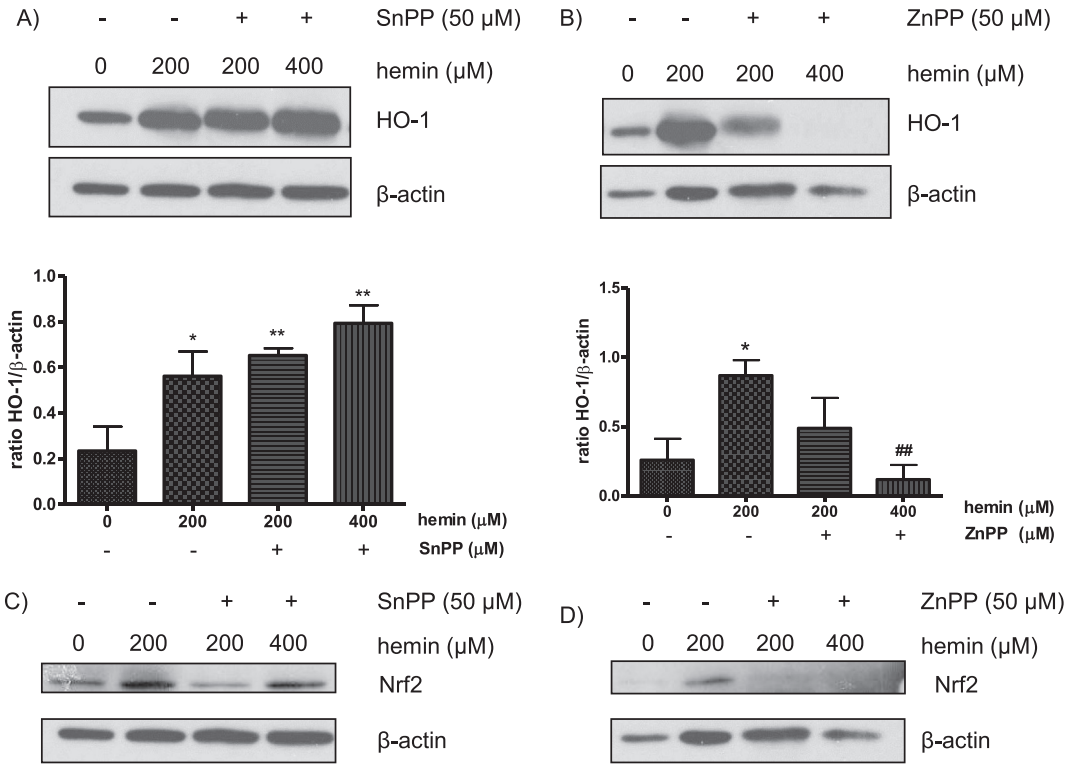

Fig. 3. SnPP and ZnPP modulate effect of hemin on HO-1 and Nrf2 expression. Glomeruli were co-incubated with increasing concentrations of hemin (200, 400 μM) and SnPP or ZnPP (50 μM) for 18 h and protein lysates were immunobloted for HO-1 (A and B) or Nrf2 (C and D). Representative western blot and mean ± SEM of densitometry values from three independent experiments are presented in the graphs *p < 0.05, **p < 0.01 vs 0 μM hemin, ##p < 0.01 vs 200 μM hemin only.

Specifically, HO-1 protein increased at CoPP concentrations 50–200 μM and decreased to near control levels at 400 μM. SnPP reduced glomerular HO-1 protein (compared to vehicle) at all concentrations tested (Fig. 2D). In contrast, ZnPP increased HO-1 protein (Fig. 2E) with the increase in response to 50 and 100 μM ZnPP being similar in magnitude to that of 200 μM hemin (used as a positive control). Both SnPP and ZnPP abolished glomerular HO-2 protein expression (Fig. 2F and G).

We next examined whether the opposite effect of SnPP and ZnPP on HO-1 could modulate the HO-1 expression pattern in response to hemin. In these 18 h co-incubation experiments, hemin was used at concentrations of 200 or 400 μM, while SnPP or ZnPP at 50 μM, a concentration 4- to 8-fold lower than that of hemin, at which SnPP or ZnPP, are not expected to competitively inhibit HO enzyme activity. As shown in Fig. 3A, in co-incubations with SnPP and 400 μM hemin, the drop in HO-1 protein observed in response to 400 μM hemin alone (Fig. 1A) was reversed. In sharp contrast, HO-1 protein markedly decreased to a barely detectable level in co-incubations with ZnPP and 400 μM hemin (Fig. 3B) and a decrease was also observed in ZnPP and 200 μM hemin (Fig. 3B). Changes in Nrf2 protein are shown in Fig. 3C and D. In co-incubations with SnPP and 400 μM hemin, the drop in Nrf2 protein observed in response to hemin alone (Fig. 1E) was reversed (Fig. 3C). In contrast, in co-incubations of ZnPP and hemin (200 or 400 μM) Nrf2 protein levels were barely detectable (Fig. 3D).

The opposite HO-1 expression patterns observed when glomeruli were co-incubated with hemin and either SnPP or ZnPP (Fig. 3A and B) support the hypothesis that intrinsic HO-1 levels (content) negatively modulate HO-1 response to exogenous heme. To provide definitive evidence for this effect, we over expressed HO-1 in glomeruli using Sleeping Beauty (SB) transposon mediated transgenesis.

3.3. HO-1 over expression negatively modulates hemin-mediated HO-1 induction

These experiments employed rats with HO-1 over expression targeted to GEC using an SB-hHO-1 vector (Fig. 4A). Organ screening for FLAG protein revealed preferential localization in the kidney with robust expression in isolated glomeruli (Fig. 4B). In glomeruli from TG rats, HO-1 protein was undetectable in 18 h incubations with 400 μM hemin (Fig. 4C). Furthermore, HO-1 protein levels following exposure to 100 and 200 μM hemin were lower than those in WT glomeruli (Fig. 4C) in a similar manner to the co-incubations of hemin and MG132 (Fig. 2B) or hemin and ZnPP (Fig. 3B).

4. Discussion

The absence of HO-1 expression in the glomerulus under conditions of injury has been convincingly documented *in-vivo* [11] and contrasts with the observation that each cell type comprising the glomerulus can upregulate HO-1 expression in response to established inducers when studied *in vitro*. The present study was prompted by this discrepancy and was performed in freshly isolated whole glomeruli as they are unique cellular entities [23] with intense cross talk between the three glomerular cell types (endothelial, mesangial, epithelial) which comprise them [24], and demonstrate different responses and behavior than the individual cell types.

Our observations demonstrate that isolated glomeruli upregulate HO-1 in response to its natural substrate, heme, as well as in response to other MPs. The increase in glomerular HO-1 expression in response to high hemin concentrations continued up to a certain “threshold”, which was reached following ~12 h incubation (Fig. 1E)

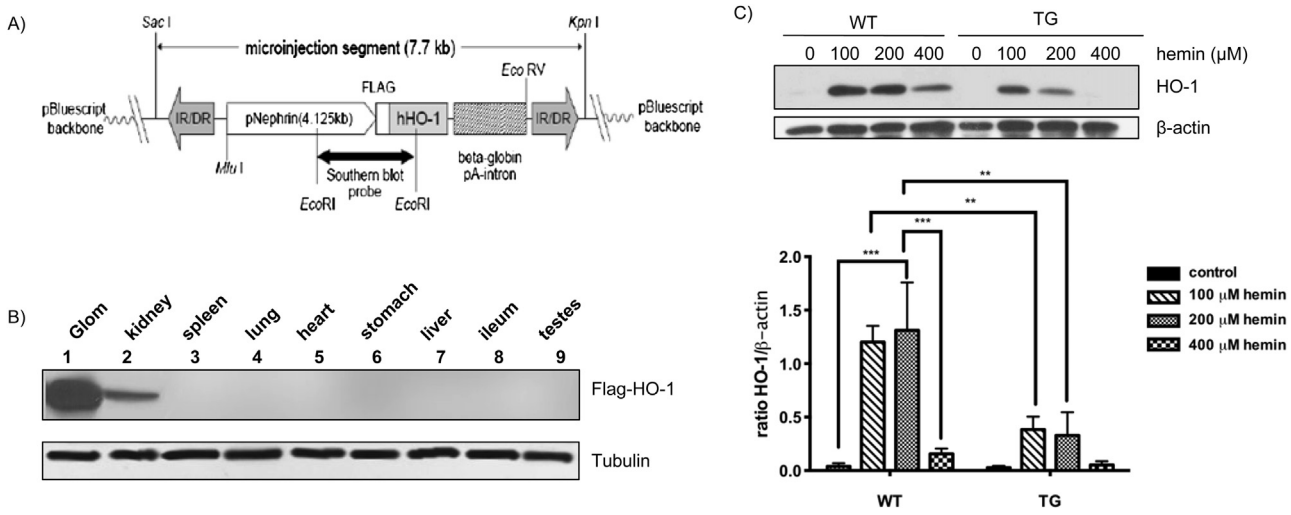

Fig. 4. HO-1 overexpression in glomeruli negatively regulates of HO-1 response to hemin. (A) SB-hHO-1 construct: A 6.9 kb DNA cassette containing tandem-arrayed murine nephrin promoter region (4.125 kb), FLAG-tagged hHO-1 and a poly (A)-intron sequence of human beta globin was inserted between two inverted repeat/direct repeat (IR/DR) sequences in SB transposon vector. (B) FLAG–HO–1 protein levels in various tissue specimens including whole kidney and isolated glomeruli. Tissues were derived from a TG rat with HO-1 overexpression targeted to podocytes. (C) Effect of hemin on HO-1 expression in glomeruli from TG and WT rats. Representative western blot and mean ± SEM of densitometry values from three independent experiments are presented in the graphs $^{**}p < 0.01$, $^{***}p < 0.001$.

and beyond which HO-1 protein levels declined to basal (Fig. 1A(ii)). The concentrations of hemin employed in these experiments are pathophysiologically relevant as they range from concentrations shown to protect against superoxide-mediated cell toxicity [20] to those found in haemolytic disorders. These include the hemolytic uremic syndrome (HUS), paroxysmal nocturnal hemoglobinuria (PNH), malaria, and various hemoglobinopathies. Free heme concentrations in these disorders can reach levels of ~230 μM, a concentration shown to correspond to lysis of >0.1% of circulating erythrocytes [21]. Exposure of glomerular cells to heme can, therefore, increase dramatically particularly in the water filtering segment of glomerular capillaries in which local heme concentration is likely to be higher than that in peripheral blood. The same may occur in various hematuric forms of glomerular injury, such as aggressive variants of gromerulonephritis, [7].

Decreased HO-1 protein expression in response to high hemin concentrations (400 μM) has been reported in a previous study [20]. Clarck et al., reported attenuation of HO-1 expression to basal levels following exposure of cultured vascular smooth muscle cells to 400 μM hemin. However, the underlying mechanism was not explored. Our observations demonstrate that the underlying factor limiting glomerular HO-1 expression is dependent on intrinsic HO-1 levels (content). This hypothesis is supported by the time course observation, in which the decrease in HO-1 at the end of the 18 h incubation (Fig. 1A(ii)) took place following a state of prolonged and sustained HO-1 expression (Fig. 1E), and the observation that raising HO-1 content by co-incubating glomeruli with hemin and either MG132 or ZnPP or by using HO-1 overexpressing glomeruli the negative modulation of HO-1 expression by hemin was observed at progressively lower hemin concentrations suggesting presence of an HO-1 "threshold" level beyond which this negative modulation is triggered.

As mentioned earlier, in clinical and experimental forms of glomerular injury increased HO-1 expression in intrinsic glomerular cells is barely detectable or undetectable. Although the present study does not elucidate the mechanism underlying the poor HO-1 expression response to injury *in vivo*, it does demonstrate that the magnitude of HO-1 response to its natural substrate/inducer is subject to negative modulation following prolonged and sustained HO-1 expression. This could be teleologically justified in situations where HO-1 reaches high expression levels with possible detrimental rather than beneficial effects owing to release of catalytically active Fe^{++}. Furthermore, the present study is the first to describe changes in HO-1 and HO-2 expression in response to specific MPs in the glomerulus. Awareness of the inhibitory effect of MPs on both HO-1 and HO-2 expression, as for SnPP (Fig. 2D and F), could be of clinical significance in haemolytic or hyperbilirubinemic disorders where systemic administration of this MP has been used as a strategy to block bilirubin production by competitively inhibiting HO. These disorders include acute or intermittent porphyrias [25] and hypebilirubinemic syndromes such as neonatal jaundice, primary billiary cirrhosis, Gilbert syndrome, and idiopathic hemochromatosis [26,27]. Inhibition of both HO-1 and the constitutive isoform, HO-2, may increase the risk of kidney injury when using MPs to treat the aforementioned disorders.

In summary, our observations identify a unique HO-1 expression pattern in the rat glomerulus and support presence of HO-1 expression control. This may prevent adverse consequences resulting from sustained HO-1 induction.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

[1] M.P. Soares, F.H. Bach, Heme oxygenase-1: from biology to therapeutic potential, Trends Mol. Med. 15 (2009) 50–58.

[2] S.F. Yet, A. Pellacani, C. Patterson, L. Tan, et al., Induction of heme oxygenase-1 expression in vascular smooth muscle cells. A link to endotoxic shock, J. Biol. Chem. 272 (1997) 4295–4301.

[3] J.M. Gutteridge, D.A. Rowley, B. Halliwell, Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts. Detection of 'free' iron in biological systems by using bleomycin-dependent degradation of DNA, Biochem. J. 199 (1981) 263–265.

[4] D.M. Suttner, P.A. Dennery, Reversal of HO-1 related cytoprotection with increased expression is due to reactive iron, FASEB J. 13 (1999) 1800–1809.

[5] U. Muller-Eberhard, J. Javid, H.H. Liem, et al., Plasma concentrations of hemopexin, haptoglobin and heme in patients with various hemolytic diseases, Blood 32 (1968) 811–815.

[6] S. Kumar, U. Bandyopadhyay, Free heme toxicity and its detoxification systems in human, Toxicol. Lett. 157 (2005) 175–188.

[7] S. Glagov, C. Zarins, D.P. Giddens, et al., Hemodynamics and atherosclerosis. Insights and perspectives gained from studies of human arteries, Arch. Pathol. Lab. Med. 112 (1988) 1018–1031.

[8] S.A. Gaertner, U. Janssen, T. Ostendorf, et al., Glomerular oxidative and antioxidative systems in experimental mesangioproliferative glomerulonephritis, J. Am. Soc. Nephrol. 13 (2002) 2930–2937.

[9] V. Jeney, J. Balla, A. Yachie, et al., Pro-oxidant and cytotoxic effects of circulating heme, Blood 100 (2002) 879–887.

[10] F.C. Fervenza, A.J. Croatt, C.M. Bittar, et al., Induction of heme oxygenase-1 and ferritin in the kidney in warm antibody hemolytic anemia, Am. J. Kidney Dis. 52 (2008) 972–977.

[11] K. Morimoto, K. Ohta, A. Yachie, et al., Cytoprotective role of heme oxygenase (HO)-1 in human kidney with various renal diseases, Kidney Int. 60 (2001) 1858–1866.

[12] M. Shimizu, K. Ohta, Y. Yang, et al., Glomerular proteinuria induces heme oxygenase-1 gene expression within renal epithelial cells, Pediatr. Res. 58 (2005) 666–671.

[13] P.K. Datta, S. Reddy, M. Sharma, et al., Differential nephron HO-1 expression following glomerular epithelial cell injury, Nephron Exp. Nephrol. 103 (2006) e131–138.

[14] S.P. Berger, M. Hunger, B.A. Yard, et al., Dopamine induces the expression of heme oxygenase-1 by human endothelial cells in vitro, Kidney Int. 58 (2000) 2314–2319.

[15] P.K. Datta, E.A. Lianos, Nitric oxide induces heme oxygenase-1 gene expression in mesangial cells, Kidney Int. 55 (1999) 1734–1739.

[16] M.J. Bek, S. Wahle, B. Muller, et al., Stra13, a prostaglandin E2-induced gene, regulates the cellular redox state of podocytes, FASEB J. 17 (2003) 682–684.

[17] P. Duann, E.A. Lianos, GEC-targeted HO-1 expression reduces proteinuria in glomerular immune injury, Am. J. Physiol. Ren. Physiol. 297 (2009) F629–F638.

[18] K. Katter, A.M. Geurts, O. Hoffmann, et al, Transposon-mediated transgenesis, transgenic rescue, and tissue-specific gene expression in rodents and rabbits, FASEB J. 27 930–941.

[19] J.E. Wiggins, S.R. Patel, K.A. Shedden, M. Goyal, B.L. Wharram, S. Martini, M. Kretzler, R.C. Wiggins, NFkappaB promotes inflammation, coagulation, and fibrosis in the aging glomerulus, J Am Soc Nephrol 21 (2010) 587–597.

[20] J.E. Clark, R. Foresti, C.J. Green, R. Motterlini, Dynamics of haem oxygenase-1 expression and bilirubin production in cellular protection against oxidative stress, Biochem J 348 (Pt 3) (2000) 615–619.

[21] A.W. Pawluczkowycz, M.A. Lindorfer, J.N. Waitumbi, R.P. Taylor, Hematin promotes complement alternative pathway-mediated deposition of C3 activation fragments on human erythrocytes: potential implications for the pathogenesis of anemia in malaria, J Immunol 179 (2007) 5543–5552.

[22] J. Alam, D. Stewart, C. Touchard, S. Boinapally, A.M. Choi, J.L. Cook, Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene, J Biol Chem 274 (1999) 26071–26078.

[23] M.W. Steffes, D. Schmidt, R. McCrery, J.M. Basgen, G. International Diabetic Nephropathy Study, Glomerular cell number in normal subjects and in type 1 diabetic patients, Kidney Int 59 (2001) 2104–2113.

[24] B.S. Haraldsson, The endothelium as part of the integrative glomerular barrier complex,, Kidney Int 85 (2014) 8–11.

[25] K.E. Anderson, J.R. Bloomer, H.L. Bonkovsky, J.P. Kushner, C.A. Pierach, N.R. Pimstone, R.J. Desnick, Recommendations for the diagnosis and treatment of the acute porphyrias, Ann Intern Med 142 (2005) 439–450.

[26] K.E. Anderson, C.S. Simionatto, G.S. Drummond, A. Kappas, Disposition of tin-protoporphyrin and suppression of hyperbilirubinemia in humans, Clin Pharmacol Ther 39 (1986) 510–520.

[27] L. Berglund, B. Angelin, R. Hultcrantz, K. Einarsson, L. Emtestam, G. Drummond, A. Kappas, Studies with the haeme oxygenase inhibitor Sn-protoporphyrin in patients with primary biliary cirrhosis and idiopathic haemochromatosis, Gut 31 (1990) 899–904.