Protective effects of tubular liver-type fatty acid-binding protein against glomerular damage in murine IgA nephropathy

Nan Zuo1,2, Yusuke Suzuki1, Takeshi Sugaya1,3, Ken Osaki1, Yasuhiko Kanaguchi1, LiNing Wang2 and Yasuhiko Tomino1

1Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan, 2Division of Nephrology, The First Affiliated Hospital of China Medical University, Shenyang, China and 3CMIC Company Limited, Tokyo, Japan

Correspondence and offprint requests to: Dr. Yasuhiko Tomino, E-mail: yasu@juntendo.ac.jp

Abstract

Background. Liver-type fatty acid-binding protein (L-FABP) in proximal tubules was reported to have renoprotective roles in experimental tubulointerstitial diseases via its anti-oxidative properties. Since tubuloglomerular cross-talk was recently discussed in the progression of renal diseases, to investigate whether tubular L-FABP may have an impact on the progression of glomerular damage, we induced IgA nephropathy (IgAN) in mice (Tg) transgenically tubular overexpressing human L-FABP (hL-FABP).

Methods. We reconstituted IgAN by bone marrow transplantation (BMT) from IgAN-prone mice into Tg and wild-type (WT) mice. Renal damage was evaluated at 6 and 12 weeks after BMT. During in vitro experiments, mesangial cells (MC) were stimulated by aggragated IgA (AIgA), and their supernatants (AIgA-MC medium) were collected. Stable cell line of mouse proximal tubular cell (mProx) transfected with or without hL-FABP gene was cultured with the AIgA-MC medium.

Results. Although mesangial IgA deposition and serum IgA level were not different between WT (WT/ddY) and Tg (Tg/ddY) recipients, WT/ddY mice showed a significantly higher urinary albumin level and mesangial matrix expansion with a significantly higher glomerular damage score. Furthermore, CD68+ macrophage infiltration was also significantly attenuated in Tg/ddY mice. Up-regulation of renal hL-FABP was associated with significant suppression of renal heme oxygenase-1 (HO-1) expression and accumulation of 4-hydroxy-2-nonenal (4-HNE) and MCP-1 expression in Tg/ddY mice. In vitro experiments showed that AlgA-MC medium and recombinant TNF-α significantly up-regulated hL-FABP expression, which was partially blocked by anti-TNF-α antibody, and major mediators of oxidative stress (HO-1 and 4-HNE) and inflammation (MCP-1). Importantly, such up-regulation of the mediators in mProx with hL-FABP was significantly suppressed much more than that in mProx.

Conclusions. Tubular L-FABP activated by MC-origin humoral factors may lessen progression of glomerular damage at early stages of IgAN by reducing oxidative stress and inflammatory mediators.

Keywords: IgA nephropathy; liver-type fatty acid-binding protein (L-FABP); oxidative stress; tubuloglomerular cross-talk

Introduction

Liver-type fatty acid-binding protein (L-FABP) is a 14.4-kDa protein expressed in human proximal tubules and known to facilitate the cellular uptake, transport and metabolism of fatty acids and regulate the expression of genes involved in lipid metabolism [1,2]. Moreover, L-FABP can bind long-chain fatty acid oxidation products with high affinity and may be a crucial cellular antioxidant molecule [3]. Because L-FABP is not expressed in the kidneys of rodents, human L-FABP (hL-FABP) chromosomal transgenic (Tg) mice were generated [4]. Several experimental models using the hL-FABP Tg mice with renal diseases, such as protein overload [4], unilateral ureteral obstruction [5] and diabetic nephropathy [6], showed that tubular overexpression of hL-FABPplays renoprotective roles via its anti-oxidative properties in such tubulointerstitial diseases. However, the pathophysiological role of L-FABP in glomerular damage of glomerulonephritis remains to be determined.

IgA nephropathy (IgAN) is defined as glomerulonephritis with predominant deposition of IgA in the glomerular mesangium [7], and frequently progresses to end-stage renal failure [8]. Although IgAN is the most common primary glomerular disease worldwide, no specific therapy is available to date, and the exact etiological mechanism remains elusive. Recent studies on the pathogenic mechanisms of IgAN have stressed the role of intra-renal oxidative stress [9]. Treatments with antioxidants such as fish-oil extracts are reported to be beneficial in both experimental [10] and human IgAN [9]. Furthermore, Kobori et al. [11] recently showed that immunoreactivities of intra-renal
heme oxygenase-1 (HO-1) and 4-hydroxy-2-nonenal (4-HNE), markers of oxidative stress, were significantly higher in IgAN patients than those in the control group. In addition, they also provided direct evidence for the role of activated renin in the early stages of experimental IgAN through interaction with the activated renin–angiotensin system (RAS) [12, 13].

The ddY mice are known to develop spontaneous IgAN [14]. Since they are not inbred, the incidence of IgAN among these mice is highly variable. Recently, we found that ddY mice could be classified into three groups: early-onset, late-onset and quiescent groups [15]. We reported that inbreeding of early-onset ddY mice for >20 generations produced a stable IgAN-onset ddY mice model [16]. Previous reports including our studies indicated that bone marrow cells (BMCs) may contribute to the pathogenesis of IgAN [17–19]. Indeed, we experimentally proved that IgAN can be replicated by BM transplantation (BMT) with the IgAN-onset ddY mice as donors in all healthy recipients [20]. In addition, this model can provide the same stage of IgAN. The present study aimed to assess the influence of tubular L-FABP on glomerular damage without influence of severe tubular damage, this is important for examining the pathophysiological process from early phase of IgAN. Recently, Ohashi et al. [13] demonstrated that the inhibition of tubular oxidative stress in an experimental IgAN model could improve glomerular damage, indicating the possibility of tubuloglomerular cross-talk in IgAN.

In the present study, we induced IgAN in hL-FABP Tg and WT recipient mice by BMT from IgAN-onset ddY mice to investigate the pathophysiological impact of tubular L-FABP on the progression of glomerular damage in IgAN.

### Materials and methods

**IgAN model**

Details of the generation of hL-FABP Tg mice have been reported previously [4]. Tg mice were backcrossed for more than six generations onto C57BL/6 mice to obtain homozygous mutant mice on an inbred background. Only female mice were used in this study. Our inbred IgAN-onset ddY mice [15] were used as donors for BMT. Twelve- to 14-week-old female Tg mice (Tg; n = 19; body weight, 22.8 ± 0.4 g; mean ± SD) and WT littermates on a C57BL/6 background (WT; n = 19; body weight, 22.1 ± 0.3 g) were used in this study. BMT was performed in WT and Tg mice as described previously by our group [20]. Both kinds of transplanted mice (WT/ddY and Tg/ddY mice) were sacrificed at 6 (n = 6 in each group) and 12 weeks (n = 10 in each group) after BMT. Normal WT (n = 3) and Tg (n = 3) mice were used as controls. Every experimental protocol with mice was approved by the Ethics Review Committee for Animal Experimentation of Juntendo University School of Medicine.

**Serum and urinary analyses**

Serum IgA concentrations were measured by Mouse IgA ELISA Quantitation Kit (BETHYL, Tokyo, Japan). Urinary albumin from samples collected over 24 h using metabolic cages was measured with an enzyme-linked immunosorbent assay kit (Albuwell, Exocell, Philadelphia, PA, USA). Urinary hL-FABP was measured by the sandwich ELISA kit for hL-FABP (CMIC Co., Tokyo) [4–6]. Intra-assay reproducibility was determined using the same sample eight times, with a coefficient of variation within 10%. This assay system does not detect L-FABP of rodents, particularly those derived from WT mice. Urinary hL-FABP was expressed as the ratio of the urinary L-FABP in nanograms to urinary creatinine in micromolars.

### Table 1. Primer sequences for quantitative real-time PCR assay

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>hL-FABP</td>
<td>Forward: 5′ AAA TCG TGC AGA ATG AGA AG 3′ Reverse: 5′ TCT CTC TCT TGA GCT TGG TGA 3′</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Forward: 5′ TTC AAA TGG GTA GCC TGG AG 3′ Reverse: 5′ CCT CTC TCT TGA GCT TGG TGA 3′</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Forward: 5′ CAA GTG CTG GAG GAC GT 3′ Reverse: 5′ CCT GCC GTT GTT CTC CAG AT 3′</td>
</tr>
</tbody>
</table>

### Histological analyses

Kidney sections (3-μm thickness) were fixed in 4% paraformaldehyde and stained with periodic acid–Schiff reagent to assess histological changes by light microscopy. The extent of glomerular damage (glomerular pathological score) was evaluated using a previously described semiquantitative scoring system with modification [21]. Briefly, we examined >30 glomeruli per animal (n = 5 in each group) and scored each aspect as follows: (i) matrix expansion: 0, absent; 1, <25% of glomeruli affected; 2, 25–50% glomeruli affected; and 3, >50% of glomeruli affected; (ii) the adhesion of the capillary tuft to the Bowman’s capsule: 0, no; and 1, yes; (iii) tuft numbers of mesangial cell (MC) proliferation determined by MC >3 in one tuft: 0, absent; 1, one tuft; 2, two tufts; and 3, >3 tufts; (iv) capillary collapse: 0, absent; 1, <25% of glomeruli affected; 2, 25–50% glomeruli affected; and 3, >50% of glomeruli affected. The expansion of mesangial matrix was estimated by the percentage of intra-glomerular area occupied by mesangial matrix in 20 glomeruli of each mouse (n = 6 in each group) with Image J (version 1.43q, NIH), and the average was used for analysis.

Glomerular infiltration of macrophages was evaluated by immunostaining in frozen section (3-μm thick) using biotinylated rat anti-mouse CD68 monoclonal antibody (Serotec). We examined >30 glomeruli in each animal (n = 3 in each group). The paraffin sections were incubated with monoclonal mouse anti-hL-FABP antibody (a gift from CMIC Co.) [4] and subsequent HRP-labeled anti-mouse antibody (Dako Cytomation, Carpentaria, CA) before using DAB kit (Dako Cytomation). The frozen sections were also used for immunofluorescence analyses with phycoerythrin (PE)-conjugated goat anti-mouse IgA antibody (BD Biosciences, Pharmingen, San Diego, CA, USA).

### Real-time quantitative PCR analysis

Total RNA was isolated by TRIZOL reagent (Life Technologies Inc., Carlsbad, CA, USA) according to the instructions provided by the manufacturer. One-microgram aliquots of total RNA were reverse-transcribed using Random Decamers (Ambion, Austin, TX, USA) and reverse transcriptase, M-MLV (Invitrogen Life Technologies, Carlsbad, CA). The products were subjected to real-time PCR using Applied Biosystems 7500 Real Time PCR System with SYBR Green Master Mix (Applied Biosystems, Tokyo, Japan) and specific primers (Table 1). The gene expression of the target sequence was normalized to that of GAPDH.

### Preparation of isolated glomeruli

The glomeruli were isolated by standard mechanical sieving technique, as previously described [22]. Briefly, kidneys were decapsulated, and sinus fat was excised. Isolated cortices were minced to a paste-like consistency with a surgical blade. The tissue paste was then forced through a series of mesh filters with 200-, 100- and 70-μm openings. Retained material on the last sieve was rinsed off and transferred to PBS at 4°C. Glomerular fractions were collected by centrifugation (1500 rpm for 5 min) and washed with PBS containing protease inhibitors.

### Western Blotting

Protein extracts were prepared from kidney tissues, isolated glomeruli (for type IV collagen and fibronectin) or cells with lysis buffer (50 mM Tris–HCl, pH 7.4, 1% sodium dodecyl sulfate–polyacrylamide gels. Monoclonal mouse anti-hL-FABP (a gift from CMIC Co.) [4] polyclonal rabbit anti-HO-1 monoclonal antibody (Serotec). We examined >30 glomeruli in each animal (n = 3 in each group) with Image J (version 1.43q, NIH), and the average was used for analysis.
Renoprotective effects of L-FABP in murine IgAN

StressGen Biotechnology (Victoria, BC, Canada), monoclonal mouse anti-4-HNE (Japan Institute for the Control of Aging, Shizuoka, Japan), polyclonal goat anti-fibronectin (Santa Cruz Biotechnology), polyclonal rabbit anti-collagen IV (Abcam Inc., Cambridge, MA, USA) and mouse anti-GAPDH (Abcam Inc., Cambridge, MA, USA) antibodies were used as primary antibodies. Blots were visualized by enhanced chemiluminescence (ECL system; Amersham Int., Buckinghamshire, UK).

Cell culture
Murine MCs were obtained from primary cell culture of C57BL/6 mice, as described previously [23]. The cells were cultured with RPMI 1640 (Sigma, St. Louis, MO, USA) supplemented with 20% FCS (Life Technologies), 100 U/mL penicillin G, 100 μg/mL streptomycin and 5 μg/mL insulin-transferrin-sodium selenite media supplement (Sigma) in a 5% CO2 environment at 37°C. MCs were identified by immunostaining with desmin, negative staining for von Willebrand factor and cytokeratin, and morphological observation. The cells survived after incubation in a medium in which L-valine was replaced with D-valine, excluding contamination with fibroblasts. MCs at passage 3 were used in the experiments.

Preparation of spent (condition) medium
Mouse IgA (Cappel, MP Biomedicals, Solon, OH, USA) were heat-aggregated by incubation at 63°C for 150 min to obtain aggregated IgA (AIgA), as described previously [26]. Growth-arrested MCs were cultured in 60-mm collagen IV-coated tissue culture dishes (IWAKI) (1 × 106 cells/dish) with RPMI 1640 medium containing 0.5% FCS and different concentrations of AIgA (final concentration was 10, 50 and 250 μg/mL) for 24 h or 10-fold diluted AIgA-MC medium for either 12 (for MCP-1 mRNA expression or collagen IV mRNA expression) or 24 h (for TNF-α mRNA expression or IL-6 mRNA expression) with or without pre-treatment by neutralizing anti-TNF-α antibody (R&D Systems, Minneapolis, MN, USA) (final concentration: 0.1–5 μg/mL) 1 h before.

Statistical analyses
All data were expressed as mean ± SD. Differences between groups were examined for statistical significance using the one-way analysis of variance (ANOVA). A P-value <0.05 was considered statistically significant.

Results
Glomerular damage in Tg mice with replicated IgAN was significantly milder than that in WT mice
Although both mesangial IgA deposition (Figure 1A) and serum elevation of IgA levels (Figure 1B) were similar between WT/ddY and Tg/ddY mice after BMT, urinary albumin level in Tg/ddY mice even at 6 weeks was significantly lower than that in WT/ddY mice (P < 0.05). Thereafter, urinary albumin level in WT/ddY mice sharply rose to 82.8 ± 65.6 mg/dL at 12 weeks, but that of Tg/ddY mice was still significantly suppressed (8.2 ± 2.2 mg/dL, P < 0.005) (Figure 2A).

Tubular hL-FABP was up-regulated in Tg mice with IgAN
Immunohistochemical analysis showed that hL-FABP expression in control Tg mice was spread profusely through the cytoplasm of the proximal tubules. In Tg/ddY mice,
immunostaining for hL-FABP showed enhanced nuclear localization of the proximal tubules (Figure 3A) and was localized more in nuclei of tubular cells at 12 weeks than that at 6 weeks. In addition, real-time PCR and western blotting analyses revealed that gene (P < 0.001) and protein (P < 0.01) expression of hL-FABP were significantly increased in the Tg/ddY kidney at 6 weeks after BMT (Figure 3B and C). Consistently, urinary hL-FABP level was also increased significantly at 6 weeks (P < 0.05) (Figure 3D).

Renal oxidative stress and inflammation were attenuated in Tg/ddY mice

HO-1 is considered to be one of the most sensitive indicators of cellular oxidative stress [27], and 4-HNE is a major aldehydic product of lipid peroxidation [28]. Both HO-1 (Figure 4A) and 4-HNE-modified protein (Figure 4B) were not detected in the control WT and Tg kidneys. Although HO-1 overexpression and accumulation of 4-HNE can be observed at 6 and at 12 weeks, respectively, their renal expression levels in Tg/ddY were significantly lower than those in WT/ddY (P < 0.05). Renal MCP-1 mRNA expression was up-regulated at 12 weeks in WT/ddY mice (P < 0.005), which was significantly higher than that in Tg/ddY mice (P < 0.01) (Figure 4C).

AIgA-MC medium enhanced the expression of hL-FABP in cultured mProx-L, partly via TNF-α

Next, we approached the underlying mechanism of renoprotection in Tg/ddY mice. We firstly analyzed the detailed mechanism of tubular up-regulation of hL-FABP in this disease. We incubated mProx-L with diluted AIgA-MC media or AIgA alone with corresponding concentration (final concentration of AIgA was 1–25 μg/mL). AIgA-MC media induced a significant up-regulation of hL-FABP mRNA and protein expression compared with control medium and was significantly more effective than AIgA itself (Figure 5A and B). The pre-incubation of neutralizing anti-TNF-α antibody with mProx-L in AIgA-MC medium can significantly suppress the up-regulation of hL-FABP at 1 μg/mL (P < 0.05) and 5 μg/mL (P < 0.01) (Figure 5C). In contrast, recombinant TNF-α itself in-
duced a significant up-regulation of hL-FABP protein expression at 50 and 250 ng/L (P < 0.005) (Figure 5D), suggesting a role of MC-origin TNF-α in tubular L-FABP expression.

**Aliga-MC medium-induced expression levels of 4-HNE and MCP-1 in mProx-L were significantly lower than those of mProx.**

To further examine the renoprotective mechanism of L-FABP, we simultaneously stimulated mProx and mProx-L with Aliga-MC medium (final concentration of Aliga was 25 μg/mL). The stimulation induced a significant up-regulation of 4-HNE-modified protein in mProx (P < 0.05), which was significantly higher than that in mProx-L (P < 0.05) (Figure 5E), and also significantly increased MCP-1 mRNA expression in both mProx (P < 0.001) and mProx-L (P < 0.001). However, the expression level of MCP-1 in mProx was ~4.7-fold higher (P < 0.001) than that of mProx-L (Figure 5F).

**Discussion**

In this study, we induced IgAN in hL-FABP Tg and WT recipients by BMT from IgAN-onset ddY donors. Although mesangial IgA deposition and serum IgA levels were similarly reconstituted, glomerular damage in Tg/ddY mice was strongly protected. Moreover, renal oxidative stress and inflammation were significantly suppressed in the Tg/ddY kidneys with up-regulation of renal hL-FABP, suggesting that renal hL-FABP may reduce oxidative stress and inflammation through inhibition of the accumulation...
We also provided experimental evidence that up-regulation of hL-FABP in the proximal tubules can be induced by humoral factors released from the glomerular mesangial cells after IgA deposition, which may be partially due to TNF-α. Overexpression of hL-FABP can suppress tubular expression of some major mediators of oxidative stress and inflammation by such humoral factors and thus may contribute to the renoprotection in Tg/ddY mice. The pathophysiological roles of renal L-FABP in kidney diseases have not yet been fully clarified. Recent animal experiments have confirmed the renoprotective role of L-FABP in tubulointerstitial disease models [4–6], in which overexpression of tubular L-FABP attenuated the tubulointerstitial damage. Although renal damage in the present model was mainly located in the glomeruli, tubular expression of hL-FABP was also significantly up-regulated in Tg/ddY mice, together with a rise of urinary hL-FABP level, suggesting that certain stimuli may act on proximal tubular cells in this glomerular disease and induce up-regulation of hL-FABP expression and subsequent renoprotection.

In IgAN, mesangial IgA deposition is one of the initial and critical events that subsequently lead to glomerular damage with a progressive clinical course. It had been demonstrated that proximal tubular epithelial cells (PTEC) do not express known specific IgA receptors [29], and IgA is rarely deposited in tubulointerstitium in IgAN [30]. IgA binding activates mesangial cells to produce inflammatory cytokines and chemokines, including TNF-α [31,32]. Normally, the glomerular barrier is impermeable to proteins, but an increase in the glomerular barrier pore size allowing the leakage of proteins into the tubular lumen has been observed in various glomerular diseases [33]. Recently, Chan et al. established a novel in vitro model to provide experimental evidence of glomerulotubular communication in IgAN: ‘spent medium’ from human mesangial cells incubated with IgA from IgAN patients induced proliferation and activation of PTEC [29]. Their finding suggests that humoral factors released from the glomerular mesangial cells maintained a glomerulotubular cross-talk in the development of IgAN. Based on the aforementioned findings, we hypothesized that up-regulation of tubular L-FABP in our IgAN model may also be due to this kind of glomerulotubular cross-talk. To confirm our hypothesis, we followed the aforementioned experimental model to stimulate mProx-L by AIgA-MC media or directly by AIgA, and found that AIgA-MC media can induce a significant up-regulation of hL-FABP gene expression and protein synthesis compared with AIgA alone. This finding suggests that certain humoral mediators released from MC after mesangial IgA deposition induced the up-regulation of L-FABP in proximal tubular cells. To confirm whether TNF-α released from mesangial cells after IgA deposition may activate renal tubular cells [29], we incubated mProx-L with neutralizing anti-TNF-α.

Fig. 3. Expression of hL-FABP in Tg/ddY kidney. (A). Representative immunostaining of hL-FABP in the kidneys (original magnification ×200). Renal hL-FABP mRNA (B) and protein (C) expression. (D). Urinary hL-FABP level expressed as a ratio to urinary creatinine (μg/g creatinine). Data are mean ± SD (*P < 0.05, **P < 0.01, ***P < 0.001).
antibody in AIgA-MC medium or recombinant TNF-α, and found that TNF-α may be a potent mediator of hL-FABP synthesis. These findings are in conflict with the results of a previous study by Memon et al. [34]. They reported that recombinant TNF-α decreased L-FABP mRNA levels in the liver of Syrian hamsters. Dubé et al. [35] also reported that TNF-α did not modify the cellular levels of L-FABP in Caco-2 cells. However, these two studies investigated different organs and cell lines from those used in our study, indicating that the expression of L-FABP may be regulated in an organ-specific manner. Further studies are needed to clarify this point. However, it is possible that IgA may directly activate tubular L-FABP in vivo. Since the nephritogenic IgA may be in polymeric or immune complex (IC), leaked polymeric or IC IgA in urine may be absorbed by specific or non-specific receptors such as megalin and thus activates tubular cells. Although present experiments with AlgA alone did not enhance L-FABP in vitro, we have to think carefully about this possibility in IgAN.

L-FABP can prevent the peroxidation of intracellular fatty acids by promoting fatty acid metabolism and by regulating gene expression involved in lipid metabolism [1,2]. Wang et al. [36] demonstrated in vitro that L-FABP may reduce oxidative stress in hypoxia–reoxygenation. Recent renal disease models of hL-FABP Tg mice also suggested that acting as an antioxidant, endogenous renal L-FABP can effectively improve the course of tubulointerstitial injury [4–6]. In our model, renal HO-1, 4-HNE and MCP-1 mRNA expression were up-regulated at 6 and 12 weeks after BMT in WT/ddY mice, respectively. All of them were significantly suppressed in Tg/ddY mice. These results indicate that oxidative stress and inflammation in murine IgAN may be attenuated by tubular L-FABP. In Tg/ddY mice, glomerular damage evaluated by mesangial matrix expansion, MC proliferation, adhesion and capillary collapsing were significantly attenuated compared with that of WT/ddY mice. Comparably, Tg/ddY mice maintained the baseline value of urinary albumin level during the experimental period, while WT/ddY mice showed significant albuminuria until 12 weeks. Moreover, glomerular infiltration of CD68+ macrophages was also significantly suppressed in Tg/ddY mice. These findings point to alleviation of glomerular damage in Tg/ddY mice. A series of previous studies stressed the pathogenic role of oxidative stress in the development of IgAN and showed that treatment with antioxidants could improve the development of IgAN [9,11,37–41]. For example, Chen et al. [37] reported that IgA-containing IC simulated the production of oxygen-free radicals by MC in situ. Kashem et al. [38] demonstrated high superoxide production by polymorphonuclear leukocytes isolated from patients with IgAN, and that such production correlated with the level of proteinuria. Descamps-Latscha et al. [39] found that high plasma levels of advanced oxidation protein products (AOPP) closely reflected a progressive form of IgAN. Recently, Kobori and co-workers [11] also found augmentation of intra-renal oxidative stress at early stages of IgAN, and that the activated intra-renal ROS played some roles in the development of IgAN by interacting with activated RAS. Trachtman et al. [40] showed that dietary treatment with antioxidant agent–vitamin E attenuated renal functional and structural changes in the experimental IgAN model. Kuemmerle et al. [41] also reported that administration of alpha tocopherol ameliorated renal injury in a rat experimental model of IgAN. Treatment with antioxidants such as fish-oil extracts in human IgAN was also reported to produce encouraging results [9]. Up-regulation of tubular L-FABP and suppression of oxidative stress of kidney were also observed in our IgAN model with subsequent amelioration of glomerular injury. Taking the aforementioned data into consideration, the present study suggests that, as an endogenous antioxidant, tubular L-FABP can improve the progression of glomerular damage in IgAN.

Ohashi et al. [13] demonstrated the association between renal damage and changes in ROS and RAS in an experimental IgAN model. Both ROS and RAS were activated...
Fig. 5. In vitro study. mRNA (A) and protein (B) expression of hL-FABP in mProx-L were up-regulated significantly by AIgA-MC medium (n = 4). (C). Up-regulation of hL-FABP in mProx-L induced by IgA-MC medium was partially blocked by neutralizing anti-TNF-α antibody (n = 4). (D). Recombinant TNF-α induced up-regulation of L-FABP (n = 3). Production of 4-HNE-modified protein (n = 4) (E) and MCP-1 mRNA expression level (n = 4) (F) in mProx-L were significantly lower than those in mProx after stimulation of AIgA-MC medium (final concentration of AIgA is 25 μg/mL). Data are mean ± SD (*P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001).
in mice with IgAN, and administration of tempol (oxygen radical scavenger) significantly suppressed the immunoreactivity of HO-1 and Ang II in the proximal and distal tubules and finally resulted in amelioration of renal damage, indicating that inhibition of oxidative stress in tubules could improve glomerular damage. It also implied the possibility of tubuloglomerular cross-talk in IgAN. Tang et al. [42] also provided compelling experimental evidence in support of the existence of a tubuloglomerular ‘cross-talk’ mechanism in the proteinuric state. After exposure to transferrin, PTEC continued to secrete PDGF, which contributed at least in part to the proliferation of MC through autocrine and/or paracrine mechanisms. Recently, we found that tubular enhancement of anti-oxidative action by L-FABP also attenuated the acute glomerular damage in acute glomerulonephritis models (anti-GBM antibody-induced glomerulonephritis) (our unpublished data). This protective effect was linked to attenuation of polymorphonuclear cell (PMN) flux in acute phase which may be due to hemodynamic change in glomeruli by tubular activation of L-FABP. The present experiment also showed significant attenuation of glomerular CD68+ macrophage infiltration in Tg mice with decrease of MCP-1 expression and oxidative stress. In addition, MCP-1 production by AlgA-MC medium in mProx-L was down-regulated significantly less than that in mProx in association with down-regulation of 4-HNE. Oxidative stress is known to modulate MCP-1 gene expression, which is, at least in part, transcriptionally regulated via activation of nuclear factor-kappaB (NF-κB) [43,44]. Although it is possible that a similar mechanism by hemodynamic alteration may exist, the present data suggests that anti-oxidative action by tubular L-FABP may reduce glomerular macrophage infiltration, at least in part, via anti-inflammatory action on MCP-1, providing protection against glomerular damage. Further studies are imperative to clarify more detailed mechanisms. Based on the results of the present study, we propose a hypothetical schema that defines the mechanisms discussed in the present study (Figure 6).

Although L-FABP is known to play a protective role in tubulointerstitial injury [4–6], we could not observe obvious tubulointerstitial damage in our IgAN model during the relatively short experimental process, which was still at the early stage up to 12 weeks after BMT and mainly showed glomerular damage. Therefore, the present model can avoid indirect influence of severe tubulointerstitial fibrosis on glomerular damage and clearly demonstrate the protective action of tubular L-FABP against glomerular damage.

In the present model, severe glomerular injury was most prominent at 12 weeks, while mRNA expression of hL-FABP and urinary level of hL-FABP at 6 weeks were higher than those at 12 weeks. This discrepancy may relate to the difference of L-FABP localization at 6 and 12 weeks, as demonstrated in Figure 3A that hL-FABP was localized more in nuclei of tubular cells of Tg mice at 12 weeks. L-FABP shuttles FFA to the nucleus, where L-FABP directly interacts with PPARα known as an anti-inflammatory mediator [45], suggesting that this shift of localization may change the efficacy of L-FABP anti-inflammatory action via PPARα transcriptional activity [46].

In conclusion, overexpression of hL-FABP in proximal tubules protected against the progression of glomerular damage of murine IgAN in association with attenuation of oxidative stress. These results suggest that suppression of oxidative stress in the proximal tubules was crucial in improving the progression of IgAN, particularly at early stages. Because the genomic DNA of hL-FABP, including its promoter region, was microinjected into fertilized eggs to generate the Tg mice, transcription of the hL-FABP gene in the Tg mice may be regulated in a manner similar to that in humans. It is possible that L-FABP in human IgAN has similar dynamics to that in our experimental IgAN model, as a self-protective system. In fact, Nakamura et al. [47] reported that urinary L-FABP was significantly higher in IgAN patients compared to both normal subjects and those diagnosed with thin basement membrane nephropathy. Thus, this study seems to provide some promising data on the improvement of progression of IgAN by agents that can

![Fig. 6. Schematic diagram of the renoprotective effect of L-FABP in murine IgAN. (A). Mechanism of progression of IgAN in WT/ddY mice: certain humoral factors from glomeruli after mesangial deposition of IgA induce oxidative stress in the proximal tubular cells (PTC), which subsequently up-regulate the gene expression of certain inflammatory mediators such as MCP-1. Oxidative stress and/or up-regulation of inflammatory mediators are transmitted to the glomerular compartment via an as yet undefined mechanism that results in aggravation of glomerular damage. (B). The renoprotective actions of L-FABP in Tg/ddY mice: TNF-α and/or other mediators released from glomeruli after mesangial IgA deposition induce up-regulation of L-FABP in PTC. The latter suppresses oxidative stress and subsequently down-regulates the expression of inflammatory mediators, which alleviates glomerular damage.](image-url)
up-regulate tubular expression of L-FABP such as PPARα agonists [48] and help design of new therapies for IgAN.

Acknowledgements. We thank Ms Shibata for her excellent technical support and Dr Tadahiro Kajiyama for management of early-onset ddY mice.

Conflict of interest statement. None declared.

References

18. van den Wall Bake AW, Daha MR, Evers-Schouten J et al. Serum IgA and the production of IgA by peripheral blood and bone marrow lymphocytes in patients with primary IgA nephropathy: evidence for the bone marrow as the source of mesangial IgA. Am J Kidney Dis 1988; 12: 410–414
Effect of chronic kidney disease on the expression of thiamin and folic acid transporters

Farhan J. Bukhari1, Hamid Moradi2, Pavan Gollapudi2, Hyun Ju Kim2, Nosratola D. Vaziri2,3 and Hamid M. Said1,2,3

1Department of Medical Research, VA Medical Center, 5901 East 7th St., Long Beach, CA 90822, USA, 2Department of Medicine and 3Department of Physiology/Biophysics, University of California School of Medicine, Irvine, CA 92697, USA

Correspondence and offprint requests to: Hamid M. Said; E-mail: hmsaid@uci.edu

Abstract

Background. Chronic kidney disease (CKD) is associated with significant cardiovascular, neurological and metabolic complications. Thiamin and folate are essential for growth, development and normal cellular function, and their uptake is mediated by regulated transport systems. While plasma folate and thiamin levels are generally normal in patients with CKD, they commonly exhibit features resembling vitamin deficiency states. Earlier studies have documented impaired intestinal absorption of several B vitamins in experimental CKD. In this study, we explored the effect of CKD on expression of folate and thiamin transporters in the key organs and tissues.

Methods. Sprague-Dawley rats were randomized to undergo 5/6 nephrectomy or sham operation and observed for 12 weeks. Plasma folate and thiamin concentrations and gene expression of folate (RFC, PCFT) and thiamin transporters (THTR-1 and THTR-2) were determined in the liver, brain, heart and intestinal tissues using real-time PCR. Hepatic protein abundance of these transporters was determined using western blot analysis.

Results. Plasma folate and thiamin levels were similar between the CKD and the control groups. However, expressions of both folate (RFC and PCFT) and thiamin (THTR-1, THTR-2) transporters were markedly reduced in the small intestine, heart, liver and brain. These events can lead to reduced intestinal absorption and impaired cellular homeostasis of these essential micronutrients despite their normal plasma levels.

Conclusions. CKD results in marked down-regulation in the expression of folate and thiamin transporters in the intestine, heart, liver and brain. These events can lead to reduced intestinal absorption and impaired cellular homeostasis of these essential micronutrients despite their normal plasma levels.

Keywords: anemia; cardiovascular disease; malnutrition; neuropathy; uremia

Introduction

Chronic kidney disease (CKD) has emerged as a major public health problem worldwide [1]. CKD is associated with an increased risk of cardiovascular disease, neurologic disorders, malnutrition and progression to end-stage renal disease. Vascular and neurologic complications in particular remain an important source of morbidity and mortality in this population [2,3].

The water-soluble vitamins are a group of structurally and functionally unrelated compounds that share the common feature of being essential for normal cellular function, growth and development. Folate and thiamin (vitamin B1)