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Selective estrogen receptor modulation attenuates proteinuria-induced renal tubular damage by modulating mitochondrial oxidative status

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Proteinuria is an independent risk factor for progressive renal diseases because it initiates or aggravates tubulointerstitial injury. Clinically, females are less susceptible to progression of chronic kidney disease; however, the mechanisms underlying the renoprotective effect of estrogen receptor stimulation have yet to be clarified. Recently, inflammasome-dependent inflammatory responses were shown to be triggered by free fatty acids, and mitochondria-derived reactive oxygen species were shown to be required for this response. Albumin-bound free fatty acids trigger inflammasome activation through mitochondrial reactive oxygen species production in human proximal tubule epithelial cells *in vitro*, an effect inhibited by raloxifene. Female ICR-derived glomerulonephritic mice (mice with hereditary nephritic syndrome) were ovariectomized and treated with raloxifene, a selective estrogen receptor modulator. Ovariectomized mice showed activation of tubular inflammasomes and elevated levels of inflammasome-dependent cytokines. Raloxifene attenuated these changes ameliorating tubulointerstitial damage, reduced production of reactive oxygen species, averted morphological changes, and improved respiratory function in mitochondria. The expression of genes that encode rate-limiting enzymes in the mitochondrial β-oxidation pathway was reduced by ovariectomy but enhanced by raloxifene. Thus, inflammasomes may be a novel and promising therapeutic target for proteinuria-induced renal injury.

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Proteinuria, a well-known marker of renal disease, is one of the risk factors for the progression of renal dysfunction, and treatment for proteinuria can help eliminate this progression as well.[1,2] Renal handling of plasma proteins involves glomerular ultrafiltration followed by tubular reabsorption. Most proteins, including albumin, appear in the glomerular ultrafiltrate, and almost all filtered proteins are reabsorbed via megalin, cubilin, or both by proximal tubular cells.[3] Usually, albumin is bound with free fatty acids (FFAs). Hence, after endocytosis, albumin is transferred to the lysosomes for degradation to amino acids, and FFAs are transferred to the mitochondria along with fatty acid-binding protein. In the mitochondria, FFAs are used for intracellular ATP production via β-oxidation, the tricarboxylic acid cycle, and electron transport system.[4,5] Excess accumulation of FFAs accelerates mitochondrial reactive oxygen species (ROS) production, leading to loss of mitochondrial membrane potential. Eventually, mitochondrial oxidative stress-induced injury occurs in renal proximal tubular cells.[6]

Men are known to be predisposed to progression of chronic kidney disease,[7,8] but the underlying mechanisms are not known. Some data suggested that estrogen receptor stimulation reduces renal injury.[9,10] Estrogen has been shown to affect mitochondrial function by reducing oxidative stress.[11,12] However, unfavorable adverse effects have limited the use of natural or synthetic estrogens in hormone-replacement therapy; hence, selective estrogen modulators (SERM) were developed. Raloxifene, a SERM, has been shown to have potentially renal benefits.[13]

We hypothesized that estrogen receptor stimulation attenuates the tubular injury induced by FFA-bound albumin by reducing mitochondrial oxidative stress. Hence, we investigated whether raloxifene could attenuate tubular damage in ICR-derived glomerulonephritis (ICGN) mice, a mouse strain with hereditary nephritic syndrome.[14] In the present study we compared renal tubular damage in ICR mice (ICR), ICGN mice (ICGN), ovariectomized ICGN mice (OVX), and OVX mice treated with raloxifene (RAL).

RESULTS

Raloxifene ameliorates tubulointerstitial damage in ICGN mice

Renal tubular damage was evaluated using Masson trichrome staining (Figure 1a–d). The extent of tubular atrophy, interstitial fibrosis, and inflammatory cell invasion in the tubulointerstitium was greater in the ICGN group than in the ICR group (Figure 1a and b). No significant differences in tubular damage were found between the ICGN and OVX groups (Figure 1c). Raloxifene improved tubulointerstitial fibrosis (Figure 1j). The tubular damage score was significantly lower in the RAL group than in the OVX group. As determined by periodic acid–Schiff staining, the glomeruli in the ICGN group showed hypercellularity, thickened capillary loops, and expanded mesangial regions, whereas those in the ICR group did not (Figure 1e and f). No significant differences in glomerular changes were observed among the ICGN, OVX, and the RAL groups (Figure 1f–h and j). The physiological data are shown in Table 1. The body weights were lower and creatinine levels were higher in the nephritic mouse groups (ICGN, OVX, and RAL) than that in the ICR group. The blood pressure tended to be higher in the ICGN and OVX groups, but no significant difference was observed. The urinary protein excretion levels did not differ among the nephritic groups (ICGN, OVX, and RAL). However, the urinary *N*-acetyl-β-D-glucosaminidase excretion level was lower in the RAL group than in the OVX group.

Raloxifene restores lipoperoxide accumulation and mitochondrial dysfunction in ICGN mice

Tubular lipoperoxide accumulation was evaluated by hexanoyl-lysine staining (Figure 2a–d). Staining was negative in the ICR group (Figure 2a). The number of hexanoyl lysine–positive tubules was higher in the ICGN and OVX groups (Figure 2b and c). Raloxifene restored lipoperoxide

Table 1 | Physiological characteristics

Group	ICR ($n=10$)	ICGN ($n=10$)	OVX ($n=10$)	RAL ($n=10$)
BW (g)	39.3 ± 3.4	28.0 ± 2.0[a]	27.8 ± 0.8[a]	23.9 ± 0.6[a]
SBP (mm Hg)	116 ± 2	123 ± 2	123 ± 3	118 ± 5
S-Cre (mg/dl)	0.16 ± 0.01	0.22 ± 0.02[a]	0.24 ± 0.02[a]	0.19 ± 0.01[a]
UPE (mg/g · U-Cre)	5.5 ± 1.3	69.1 ± 22.4 [a]	80.7 ± 8.4[a]	58.1 ± 5.9[a]
U-NAG (U/l)	9.4 ± 1.2	56.5 ± 7.5[a]	56.6 ± 3.6[a]	10.9 ± 4.5[b]

Abbreviations: BUN, blood urea nitrogen; BW, body weight; SBP, systolic blood pressure; S-Cre, serum creatinine; U-Cre, urinary creatinine; U-NAG, urinary *N*-acetyl-β-D-glucosaminidase; UPE, urinary protein excretion.
Data are expressed as mean ± s.e.m.
[a]$P<0.05$ vs. ICR.
[b]$P<0.05$ vs. OVX.

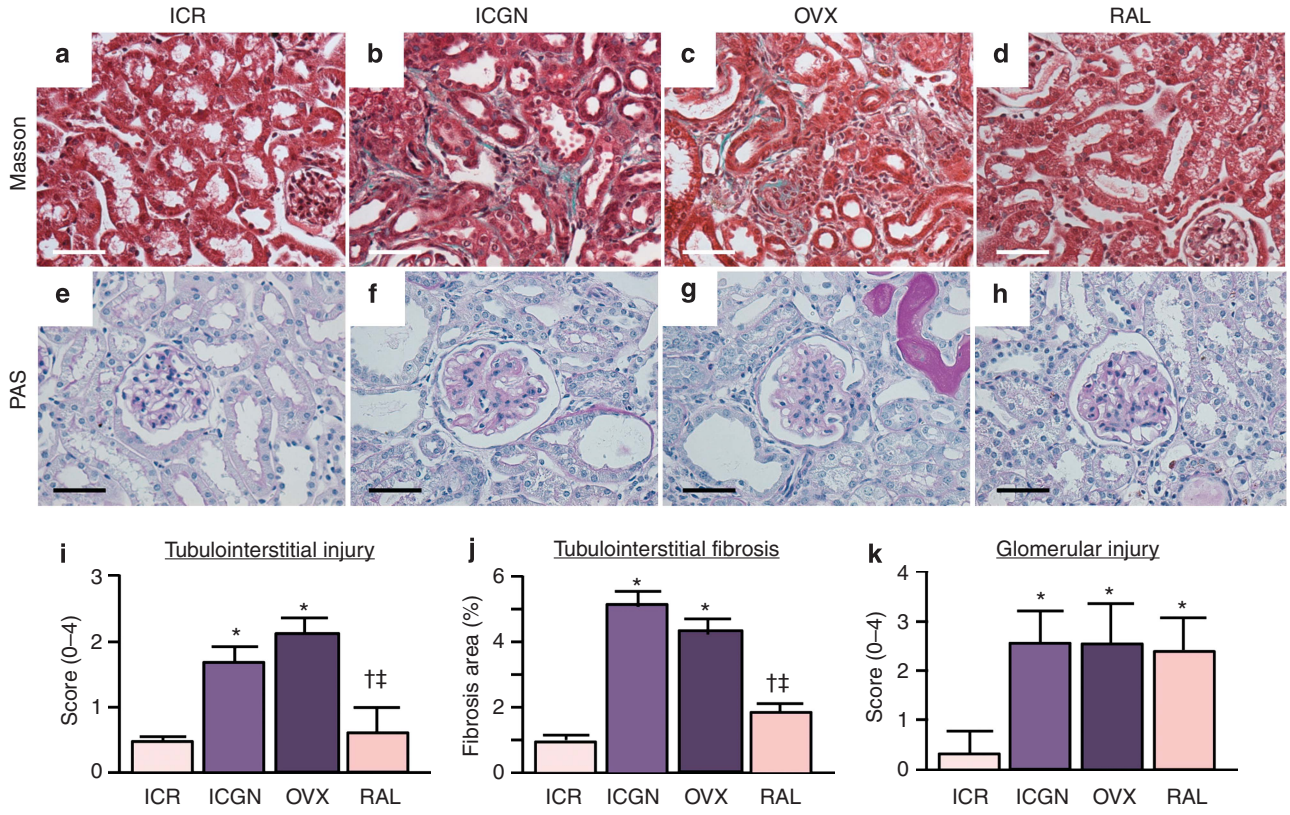

Figure 1 | Pathological changes in the tubulointerstitium and glomeruli (**a-d**) Masson-trichrome (Masson) staining showing renal tubulointerstitial morphology. Bar = 50 μm. (**e-h**) Periodic acid–Schiff (PAS) staining showing glomerular morphology. Bar = 50 μm. ICGN, ICR-derived glomerulonephritis mice; ICR, ICR mice; OVX, ovariectomized ICGN mice; RAL, OVX mice treated with raloxifene. (**i**) Tubulointerstitial injury score, (**j**) tubulointerstitial fibrosis score, and (**k**) glomerular injury score; $n=10$ in each group. Data are shown as mean ± s.e.m. *$P<0.05$ vs. ICR. †$P<0.05$ vs. ICGN. ‡$P<0.05$ vs. OVX.