Estrogens and Progression of Diabetic Kidney Damage

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Abstract: It is generally accepted that estrogens affect and modulate the development and progression of chronic kidney diseases (CKD) not related to diabetes. Clinical studies have indeed demonstrated that the severity and rate of progression of renal damage tends to be greater among men, compared with women. Experimental studies also support the notion that female sex is protective and male sex permissive, for the development of CKD in non-diabetics, through the opposing actions of estrogens and testosterone. However, when we consider diabetes-induced kidney damage, in the setting of either type 1 or type 2 diabetes, the contribution of gender to the progression of renal disease is somewhat uncertain. Previous studies on the effects of estrogens in the pathogenesis of progressive kidney damage have primarily focused on mesangial cells. More recently, data on the effects of estrogens on podocytes, the cell type whose role may include initiation of progressive diabetic renal disease, became available.

The aim of this review will be to summarize the main clinical and experimental data on the effects of estrogens on the progression of diabetes-induced kidney injury. In particular, we will highlight the possible biological effects of estrogens on podocytes, especially considering those critical for the pathogenesis of diabetic kidney damage.

Keywords: Estrogens, Chronic kidney disease, Diabetic kidney damage, Podocytes, Apoptosis.

The influence of estrogens on the development and progression of diabetic kidney damage, although a subject of intense translational research effort, remains a matter of controversy [1-5]. The aim of this review will be to summarize the clinical and experimental data regarding the effects of estrogens on the development and progression of diabetes-induced kidney damage, with special regards to the most recent data concerning podocyte injury and dysfunction.

It is generally accepted that estrogens affect and modulate the development and progression of chronic kidney diseases (CKD) not related to diabetes [1, 4, 5]. Several clinical studies have indeed demonstrated that the severity and rate of progression of renal damage tend to be greater among men, compared with age-matched women, independently of the presence of other causative factors such as hypertension, dietary protein intake, circulating lipid levels, and, in the majority of cases, etiology of renal disease [4-6]. This is, for instance, true for membranous nephropathy [7], IgA nephropathy [8], and polycystic kidney disease [9]. However, other studies have also reported that gender difference has little or no influence on the progression of renal diseases [6, 10-12], and that the progression of kidney damage may be even faster in postmenopausal women [11].

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Experimental studies also support the notion that female sex is protective, and male sex permissive, for the development of CKD in non-diabetics, through the opposing actions of estrogens and testosterone [4, 5]. For instance, data from our laboratory showed that estrogen deficiency accelerates the progression [13], and 17β-estradiol (E_2) replacement retards the development of glomerulosclerosis (GS) in ovariectomized sclerosis-prone ROP Os/+ mice [14]. In contrast, female mice transgenic for an ERα gene deletion (αERKO) develop GS because of their elevated blood testosterone levels (8-times higher than those of their female littermates) [15]. Indeed, ovariectomy prevents the onset of glomerular dysfunction in female αERKO mice by eliminating their endogenous testosterone production [15]. In contrast, testosterone supplementation induces GS in ovariectomized B6 mice, whereas estrogen deficiency following ovariectomy had no deleterious effects on the glomerulus of B6 mice [15].

The uncertainty of the contribution of gender to the progression of renal disease is even greater when we consider diabetes-induced kidney damage, in the setting of either type 1 or type 2 diabetes [2, 3]. Several clinical studies indicate that diabetic kidney damage progresses faster in males than in females [16-22]. However, some studies show that female sex accelerates the disease progression [23-26], while others report no difference in the incidence and/or rate of progression of renal disease between men and women [27-29].

For instance, male sex appeared to be a risk factor for the development of micro- and macro-albuminuria in a study on

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normotensive type 1 diabetic patients with either absent or mild CKD [21, 22], as well as in an other study on patients with established diabetic nephropathy [17]. On the contrary, no sex differences were reported in a randomized trial of captopril in type 1 diabetic patients after a 3-year follow-up [27, 30], or in a prospective study regarding the effects of smoking [31], and in a Danish study on normoalbuminuric type 1 diabetic patients followed for 10 years [29]. Finally, in some studies female sex appears to be a risk factor for the development of diabetic nephropathy in type 1 diabetes, although many of these studies were performed in children or pubertal patients, a fact that could affect and limit the validity of their conclusions [23-26].

Similar to type 1 diabetes, conflicting data have also been reported in type 2 diabetes. Most of the studies described a greater prevalence of albuminuria in male compared to female patients [32-34], or an increased risk for the development of diabetic nephropathy associated with male sex [35]. However, renal disease was reported to progress faster in females than in males in two large randomized trials [36, 37], although the inclusion in these studies of many women of postmenopausal age could have played a significant role in the outcome of these results. More conclusive data was derived from studies in African, Americans, Hispanics, and Pima Indians [38-40]. In these populations, women appear at greater risk for developing end-stage renal disease associated with type 2 diabetes than men [38-40].

Few interventional studies have tested the effects of estrogen supplementation on the progression of diabetic kidney damage. Administration of estrogens together with a synthetic progestin reduced proteinuria and improved creatinine clearance in postmenopausal women with type 2 diabetes [41], and prevented the development of albuminuria in the Insulin Resistance Atherosclerosis Study [42]. Analogously, the selective estrogen-receptor modulator raloxifene attenuated the progression of albuminuria in postmenopausal women with type 2 diabetes [43]. However, an association between estrogen treatment, alone or in combination with progestin, and increased risk for microalbuminuria has been reported in pre- and post-menopausal women [44], whereas no change on microalbuminuria caused by prolonged hyperglycemia was induced by 6-month treatment with estrogen and medroxyprogesterone in postmenopausal women with type 2 diabetes [45]. Furthermore, the use of oral contraceptives containing high concentrations of estrogens, but not of those containing low doses, has been linked to the development of albuminuria in women with type 1 diabetes [46, 47].

In experimental studies, E_2 supplementation, either from the onset or after several weeks of untreated diabetes, exerts a protective effect on the development of functional (albuminuria) and structural (GS and tubulointerstitial fibrosis) kidney damage [48-52]. These effects are mediated by different cellular mechanisms, including reduction of TGF-β synthesis, decreased accumulation of collagen type IV, laminin, and fibronectin, and increased production of matrix metalloproteinases (MMPs) [48-52]. Also raloxifene has been shown to diminish albuminuria, GS and tubulointerstitial fibrosis *via* similar effects [53, 54]. It has been also reported however, that E_2 has no effect on albuminuria in OLEFT rats [55], or that it exacerbates diabetic renal disease in sucrose-fed diabetic rats [56], suggesting that E_2 effect may depend on the disease model examined, the timing of treatment, and the dosage. Interestingly, female αERKO mice, which spontaneously develop GS due to inappropriate amounts of testosterone [15], are protected from the development of albuminuria and GS induced by experimental diabetes [57], further stressing the importance of better understanding the biological actions of sex hormones in the specific setting of diabetes.

The studies on the cellular effects of estrogens in the pathogenesis of progressive kidney damage, in our laboratory and others, have primarily focused on mesangial cells, for years considered the key player in the events leading to the progression of renal injury. In contrast, little data are available on the effects of estrogens on podocytes [52], the cell type whose role may include initiation of progressive diabetic renal disease [58-62]. The ability of the kidney to replace damaged or lost podocytes is limited since podocytes exhibit a reduced potential to regenerate *via* mitosis in the glomerulus [59, 63]. Thus, progressive podocyte damage characterized by foot process effacement, vacuolization, and detachment of podocytes from the glomerular basement membrane which finally leads to the irreversible loss of podocytes are now considered important, if not essential, initial events in the development and progression of diabetic GS [58-63]. Following we will highlight the possible biological effects of estrogens on podocytes, with particular regards to the events critical for the pathogenesis of diabetes-induced kidney damage.

REGULATION OF PODOCYTE ESTROGEN RECEPTORS BY E_2

The physiologic effects of estrogens are mediated by two distinct estrogen receptor (ER) subtypes, ERα and ERβ [64, 65]. ER expression has been localized on podocytes by histochemical studies [66]. Our laboratory confirmed by mRNA and western analysis the expression of ERα and ERβ subtypes [52]. Previous studies found that total kidney and mesangial cell ERα expression are regulated by the level of estrogens [14, 67-69]. This appears to be the case for podocyte ERβ expression. In podocytes isolated from diabetic mice treated with E_2, neither ERα copy number nor protein expression was regulated by treatment. We did, however, find an increase in ERβ protein expression without a change in ERβ mRNA copy number, suggesting a post-translational regulation, such as protein stabilization [70, 71]. As ERβ expression regulates apoptosis and cell cycle in breast cancer cells [72], the increase in the ERβ protein expression in podocytes could lead to cell cycle changes and increased cell survival, an effect which could protect against podocyte depletion in diabetic kidney injury.

E_2 EFFECT ON PODOCYTE APOPTOSIS

Podocytes are terminally differentiated cells with a limited capacity to re-enter the cell cycle and proliferate. In diabetes-induced kidney injury, a reduction in podocyte density appears to be a critical determinant for the development of proteinuria and the progression of kidney dysfunction [73]. Studies using diabetic murine models suggest that apoptosis

of podocytes leads to a reduction in the density of podocytes [73]. Podocyte apoptosis is mediated by multiple signaling pathways (see recent review by Chuang *et al.*) [74], including the activation of p38 mitogen-activated protein kinase (MAPK) and TGF-β1. Phosphorylation of p38 leads to the activation of the apoptosis machinery in podocytes. Advanced glycation end products (AGEs) activate p38 and trigger the apoptosis of podocytes in a FOXO4-dependent manner and may also activate TGFβ. In contrast, activation of phosphoinositide 3-kinase (PI3K) and its downstream target AKT (protein kinase B) protects against podocyte injury/apoptosis. Although it is well established that estrogens can inhibit apoptosis in a variety of cells and tissues, the mechanisms underlying this are not clearly understood. We and others have proposed that the regulation of signaling pathways, including those described above, by estrogens could protect against podocyte apoptosis. Data generated in our laboratory showed that E_2 treatment protects podocytes from apoptosis induced *in vitro* by TGFβ and TNF-α [75]. This effect may be mediated by activation of the PI3K-AKT signaling cascade, since podocytes isolated from diabetic mice treated with E_2 have increased levels of AKT phosphorylation (unpublished data). Moreover, when we studied db/db mice at the onset of albuminuria (12 weeks-old), we found that glomeruli of db/db mice show reduced AKT phosphorylation compared to db/+ mice [76]. In addition, podocytes isolated from db/db mice with diabetes at the onset of albuminuria, even if cultured in normal-glucose medium, showed impaired insulin-dependent AKT phosphorylation, which is associated with enhanced susceptibility to cell death [76]. Finally, since mitochondrial respiratory chain (MRC) derived reactive oxygen species (ROS) can trigger apoptosis, estrogens could potentially decrease ROS induced events by regulating podocyte antioxidant levels including Mn-superoxide dismutase (MnSOD) and glutathione (GSH) [77]. These experiments and other similar studies are ongoing in our laboratory.

REGULATION OF CYTOKINES AND SIGNALING PATHWAYS BY E_2 TREATMENT

There is mounting evidence that TGFβ promotes diabetic GS in part by mediating apoptosis and depletion of podocytes [78-81]. Treatment of diabetic mice with an anti-TGFβ antibody shortly after the onset of diabetes reduces albumin excretion and protects against podocyte loss [82]. In addition, Niranjan *et al.* identified a Notch1-dependent activation of p53 leading to TGFβ-induced podocyte apoptosis in a murine model of type 1 diabetes-induced kidney damage [83, 84]. TGFβ is also activated in podocytes by ROS, which accumulate when podocytes are exposed to AGEs and hyperglycemia [85].

TGFβ activates multiple signaling pathways, both Smad-dependent and -independent [86-88]. Based on our data, we propose that estrogens regulate many of these pathways and thereby protect against the deleterious effects of TGFβ receptor activation. E_2 has been shown in breast cancer cells to inhibit Smad3 transcriptional activity through Ap-1 transcription factors in an ER-dependent manner [89]. Whether this is the case in podocytes remains to be explored. In our study, we found that E_2 treatment of isolated podocytes decreased the activation of ERK, another downstream signaling pathway in TGFβ activation [52]. These data are consistent with earlier immunohistochemical findings that activated ERK is present in podocytes of diabetic kidneys, and can be correlated with the severity of glomerular lesions [90]. We also found a decrease in glomerular TGFβ mRNA. Although podocyte production of TGFβ has not been documented, the reduction in glomerular TGFβ could also prevent podocyte ERK activation. Since the ERK signaling pathway has been shown to cross talk with Smads and enhance collagen type I expression in human mesangial cells [91, 92], it is possible that a similar pathway occurs in podocytes.

REGULATION OF PODOCYTE MMPS BY ESTROGENS

Matrix metalloproteinases (MMPs) are crucial for maintaining the balance between extracellular matrix synthesis and degradation [93]. Specifically, MMP-2 and MMP-9 degrade type IV collagen, one of the major components of the glomerular basement membrane (GBM). Preserving the balance between MMPs and collagen is critical since alterations of the GBM could result in microalbuminuria, subsequent macroproteinuria, and eventual renal failure [94]. In glomeruli [93] and mesangial cells, MMP-2 activity and transcriptional activation is regulated by continuous *in vivo* E_2 treatment [14]. Maric and colleagues reported that kidney MMP-2 activity is upregulated after E_2 treatment in a rat model of type I diabetes [48]. Our study in type 2 diabetic mice shows that E_2 treatment participates in the remodeling of the diabetic GBM through MMP production, as collagen and laminin deposition were reduced in the glomeruli of treated *db/db* mice [52]. A recent study using podocytes isolated from non-diabetic mice showed that MMP-2 and -9 activity were enhanced by in vitro administration of TGFβ, suggesting that TGFβ might increase degradation of the GBM [95]. In contrast, our study in db/db mice revealed that podocytes isolated from the E_2 treated mice had increased MMP-2 and MMP-9 activity at the same time that TGFβ mRNA was decreased. It is possible therefore that in our model the increase in MMPs prevents GBM thickening by preserving matrix composition.

A study performed by Bai *et al.* on podocytes isolated from non-diabetic mice revealed that high glucose levels modulated MMP-9 activity and (α5) type IV collagen secretion [96]. This was in part mediated by the glucose induction of ERK1/2 and the transcriptional factor Ets-1. Since our data suggest that estrogens can regulate ERK activation as discussed above, this may be one of the signaling pathways regulating MMP-9 in our model.

E_2 EFFECT ON PODOCYTE CYTOSKELETON

Podocytes are specialized cells responsible for maintaining the selective filtration barrier of the renal glomerulus. Podocytes consist of a cell body, major processes and foot processes (FP). The FP surrounds the glomerular capillary wall and form specialized intercellular junctions, the slit diaphragm (SD) protein complex. In addition, there are apical and basal membrane domains [74, 97]. The submembranous regions of all three compartments are linked to each other through the actin cytoskeleton. Disruption of any of the three domains or the underlying actin cy-

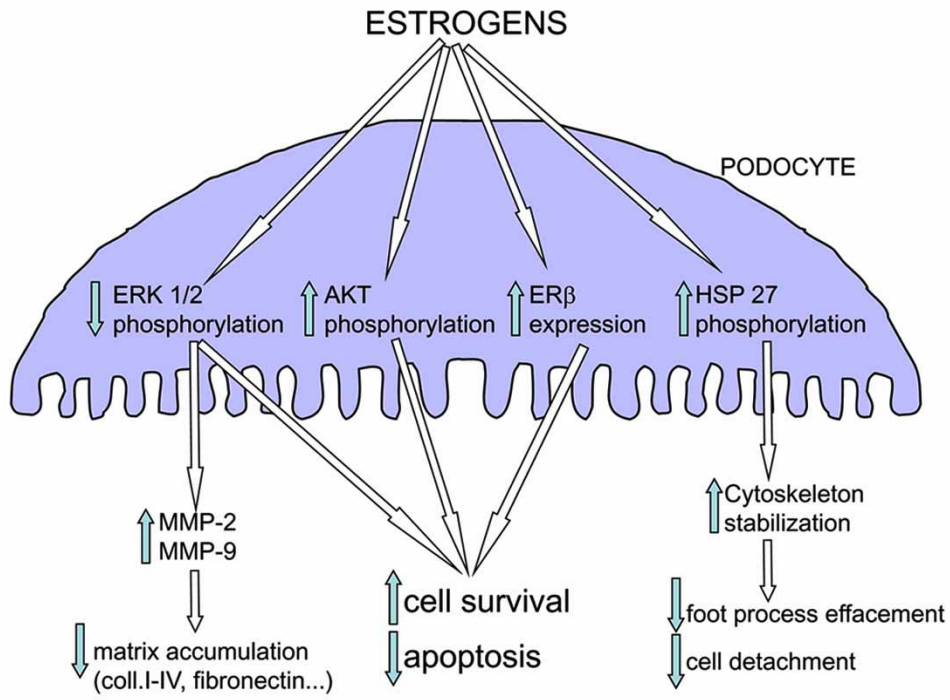

Fig. (1). Schematic representation of the signaling pathways and of the molecular mechanisms that are potential targets of estrogen action in podocytes.

toskeleton can lead to FP effacement and disruption of the glomerular filtration barrier [98, 99] The signaling molecules and pathways involved in the regulation of podocyte actin cytoskeleton were recently reviewed by Faul *et al.* [98].

As discussed above, injury of podocytes contributes to the development of diabetic kidney damage [62, 73, 100]. Recent studies have demonstrated that stress proteins may be induced and may be involved in the modulation of podocyte injury [101, 102]. In particular, HSP27, a stress protein involved in actin polymerization, is localized in podocytes [101]. HSP27 preserves actin structure, and facilitates survival in an injury environment [101, 102]. Increased activation of glomerular p38MAPK is associated with decreased phosphorylation of HSP27, changes in actin cytoskeleton, podocyte effacement, and proteinuria [101, 102]. E_2 has been shown to induce expression and phosphorylation of HSP27 in the brain and breast cancer cells [103, 104], however, to date there are no data on the regulation of HSP27 by estrogens in podocytes. Since podocyte damage and albumin excretion are decreased in a model of type 2 diabetes after E_2 supplementation [52, 53] we propose that E_2 treatment may prevent the decrease in HSP27 phosphorylation and the subsequent downstream events.

In summary: The role of estrogens in progression of diabetic kidney damage is still a matter of controversy and a subject of intense investigation. We outlined the main clinical and experimental data available, with particular regards to the possible biological effects of E_2 on podocytes, the cell type that the most recent studies indicated as crucial for the initiation of progressive diabetic renal disease.

(Fig. **1**) depicts some of the signalling pathways and of the molecular mechanisms that are potential targets of estrogen action in podocytes. Further studies will be needed gen action in podocytes. Further studies will be needed to fully elucidate the effects of estrogens in the progression of diabetes-induced kidney damage.

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