Renal and Cardiovascular Protective Effects of Estradiol Metabolites – Preclinical Evidence for Clinical Investigation

S. P. Tofovic¹, E. K. Jackson ²

Center for Clinical Pharmacology, Departments of ¹Medicine and ²Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Problem Area

Chronic renal disease (CRD) is characterized by accelerated arteriosclerosis and an excessive rate of cardiovascular morbidity, and cardiovascular disease (CVD) is recognized as the leading cause of mortality in patient with end-stage renal disease (1-3). Furthermore, even mild to moderate chronic renal disease carries high risk for cardiovascular disease (4). Despite its high prevalence, in the past little attention has been paid to cardiovascular disease in CRD patients (5). Importantly, a growing body of evidence indicates that processes contributing to CVD commence early in the progression of CRD, and suggests that early intervention may reduce the burden of cardiovascular disease in the CRD population. Although substantial experimental and clinical work has proven that estrogens are cardioprotective (6), little is known regarding the effects of estrogens in chronic renal disease. Recent data indicate that the rate of progression of chronic renal disease of various etiologies is more rapid in men than in women and is independent of blood pressure or serum cholesterol levels (7,8). Although the involvement of genetic factors, environment and androgens should not be neglected, the resistance of kidneys in women to the progression of renal disease is most frequently attributed to estrogens.

Limited renoprotective effects of estradiol

There are several mutually non-exclusive mechanisms that may account for the protective effects of 17β-estradiol against the progression of cardiovascular and renal disease (9). These include desirable effects of estradiol on plasma cholesterol levels, antioxidant effects of estradiol and its inhibition of the renin-angiotensin system. Furthermore, by altering the release of endothelium-derived factors such as nitric oxide, prostaglandins and endothelin-1, estradiol modulates vascular tone (9) and inhibits mesangial cell proliferation and extracellular matrix expansion, which are involved in initiation/progression of glomerulosclerosis (9). Although the experimental and clinical data suggest that estrogens are renoprotective, there are several limitations for using the hormone replacement therapy for renoprotection. (i) First, because of its estrogenic-feminizing effects, therapy with estradiol would be of limited value in men with chronic renal disease; (ii) Second, estradiol increases plasma triacylglycerides (TGs) in women and patients with nephrotic syndrome, and in experimental nephropathy, by further increasing TG levels, estradiol worsens renal disease (10-12); (iii) Third, because of increased risk for development of cancer in target organs, therapy with estradiol would be of limited value in pre-menopausal women; (iv) Finally, recent large prospective clinical studies (HERS Study and Women’s Health Initiative Study-WHI study; 13,14), questioned the efficacy of estrogens in attenuating cardiovascular disease and underscored the need to clarify the mechanisms of cardiovascular protection by estradiol. Obviously, the use of estradiol analogs with no estrogenic activity that exhibit renal and cardiovascular protection would provide powerful new weapons for fighting high cardiovascular morbidity and mortality in CRD population. Our recent data suggest that this may be the case with metabolites of 17β-estradiol.

Concept of renal protection by estradiol metabolites

Recent in vitro studies conducted by our group and by others (for review see refs 9,15) provide a line of evidence indicating that several of the cellular effects of 17β-estradiol (E2) are largely mediated by its downstream metabolites (15). The major metabolic pathway for estradiol includes C2 hydroxylation that leads to the formation of 2-hydroxyestradiol (2-HE). This metabolite has little estrogenic activity and is quickly (t1/2=90) converted (by COMT) to 2-methoxyestradiol (2ME), a major estradiol metabolite with no estrogenic activity. 2-Methoxyestradiol has a 500- and 3500-times lower affinity than estradiol for estrogen receptor (ER) and ERβ, respectively, whereas 2HE retains some binding activity. Estradiol metabolites, once held to be inactive, are even more potent antimitogens than estradiol itself (15). Estradiol metabolites in dose-dependent manner inhibit proliferation of vascular smooth muscle cells, fibroblasts and mesangial cells, and inhibit collagen synthesis (15, 16). 2-Methoxyestradiol is also more potent than estradiol in increasing NO and prostacyclin synthesis and in inhibiting the endothelin synthesis (15). These effects of 2ME are not blocked by specific estrogen receptor antagonists (15), suggesting the involvement of estrogen receptor-independent mechanisms. The fact that the metabolites of estradiol (i) have greater antigrowth and anti-oxidant effects; (ii) lack estrogenic activity; and (iii) have no carcinogenic effects, suggest that they may provide better cardiovascular and renal protection than estradiol itself. Our studies in rodent models of renal and cardiovascular disease indicate that estradiol metabolites indeed exert remarkable renal and cardiovascular protection.
Protective effects of estradiol metabolites in rodent models of renal and cardiovascular disease

Initially, we conducted a study with 2-hydroxyestradiol (16) in male, obese (fa-fa), diabetic ZSF1 rats, a genetic model of obesity and the metabolic syndrome, with a high risk for renal disease (17,18). Chronic administration of 2HE (10 g/kg/h) provided significant renoprotection, as evidenced by decreased proteinuria, glomerulosclerosis and severity of tubulointerstitial changes (16). However, since 24-week treatment with 2HE induced a significant reduction in food consumption and body weight, significantly decreased elevated plasma cholesterol levels and improved glucose control, it is possible that the renoprotective effects of 2-HE were due to the improved metabolic status, rather than to direct renal effects.

Therefore, in the next study (19), we examined the potential direct renoprotective effects of 2-hydroxyestradiol in chronic puromycin aminonucleoside (PAN)-induced nephropathy, a model that resembles human glomerulosclerosis. Chronic treatment with 2HE significantly attenuated PAN-induced decrease in glomerular filtration, reduced proteinuria and the elevated blood pressure and had no effects on PAN-induced increase in plasma cholesterol and triglycerides levels. The fibrotic and proliferative responses (collagen IV and PCNA) in glomeruli were significantly higher in PAN nephropathic rats versus control animals with intact kidneys. Puromycin also markedly increased glomerular and interstitial macrophage infiltration (ED1+ cells). 2-Hydroxyestradiol significantly reduced glomerular PCNA and collagen IV and glomerular and interstitial macrophage infiltration. Importantly, 2HE had no effects on plasma testosterone levels in male, nephropathic animals. This study provide the first evidence that 2HE exerts direct renoprotective effects in vivo.

The goal of the recent study (20) was to determine whether 2ME, a major non-estrogenic metabolite of estradiol and its metabolic precursor 2HE have cardiovascular and renal protective effects in vivo. We used a rat model of renal and cardiovascular injury induced by chronic nitric oxide synthase (NOS) inhibition. The prolonged 5-week NOS inhibition by N -Nitro-L Arginine (LNNA) induced severe hypertension, proteinuria, and marked reduction in glomerular filtration rate (GFR). 2-Methoxyestradiol, but not 2HE (both metabolites given at 10 g/kg/h), significantly decreased elevated blood pressure and attenuated the reduction in GFR. 2HE delayed the onset of proteinuria, whereas 2ME prevented LNNA of inducing proteinuria. Prolonged NOS inhibition resulted in high mortality (75%, LNNA group), and 2HE and 2ME reduced mortality rate by 66% and 83%, respectively. In the kidney, 2HE and 2ME abolished LNNA-induced interstitial and glomerular macrophage influx (ED1+ cells) and attenuated LNNA-induced glomerular collagen IV synthesis. Both metabolites inhibited glomerular and tubular cell proliferation PCNA), with 2ME exhibiting greater antiproliferative effects. In the heart, 2HE and 2ME markedly reduced vasculitis and interstitial inflammation and reduced collagen synthesis and vascular/interstitial cell proliferation. This study provided the first evidence that, in a model of accelerated and severe cardiovascular and renal injury, 2-methoxyestradiol exerts renal and cardiovascular protective effects and reduces mortality.

Very recently, we were able to demonstrate that estradiol metabolites have protective effects in yet another model of cardiovascular and renal injury (21). Chronic administration of angiotensin II (Ang II, 200 ng/min) induced hypertension and cardiac hypertrophy, significantly increased urinary protein excretion and reduced creatinine clearance. 2-Methoxyestradiol (10 g/kg/h) but not 2HE (10 g/kg/h), significantly attenuated Ang II-induced hypertension and cardiac hypertrophy, whereas both metabolites attenuated proteinuria.

Finally, experiments conducted in adult, obese male ZSF, rats that fully express metabolic syndrome and progressive nephropathy demonstrated that 2-methoxyestradiol and its synthetic analog 2-ethoxyestradiol (2EE), have direct renoprotective effects and retard the progression of disease in nephropathy associated with metabolic syndrome and obesity (22). Nine-week treatment with 2ME or 2EE (both at 17 g/kg/h) had no effects on food consumption and oral glucose and insulin tolerance test. Nevertheless, both 2ME and 2EE prevented time-dependent increase in proteinuria, and 2EE, reduced blood pressure. 2-ethoxyestradiol, and to a lesser extent 2ME, significantly increased renal blood flow and glomerular filtration, and significantly reduced renal vascular resistance. 2-Ethoxyestradiol and 2ME also reduced the increased protein expression of markers of proliferation and inflammation (PCNA, and NF-kappa B) in renal cortical tissue.

In summary, the presented data provide preclinical evidence for, and warrant clinical investigation of cardiovascular and renal protective effects of estradiol metabolites in patients with chronic renal disease.

References


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