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Endothelin system expression in the kidney following cisplatin-induced acute kidney injury in male and female mice

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Abstract

The chemotherapeutic agent cisplatin accumulates in the kidney and induces acute kidney injury (AKI). Preclinical and clinical studies suggest that young female mice and women show greater recovery from cisplatin-AKI compared to young male mice and men. The endothelin (ET) and ET receptors are enriched in the kidney and may be dysfunctional in cisplatin-AKI; however, there is a gap in our knowledge about the putative effects of sex and cisplatin on the renal ET system. We hypothesized that cisplatin-AKI male and female mice will have increased expression of the renal ET system. As expected, all cisplatin-AKI mice had kidney damage and body weight loss greater than control mice. Cisplatin-AKI mice had greater cortical *Edn1*, *Edn3*, *Ednra*, and *Ednrb*, while outer medullary *Ednra* was significantly suppressed in both sexes. Of the ~25 000 genes sequenced from the inner medulla, only 91 genes (comparing saline mice) and 134 genes (comparing cisplatin-AKI mice) were differentially expressed and they were unrelated to the ET system. However, *Edn1* was significantly greater in the inner medulla of male and female cisplatin-AKI mice. Thus, RNA profiles of the ET system were significantly affected by cisplatin-AKI throughout the kidney regardless of sex and this may help determine the therapeutic potential of targeting the ET receptors in cisplatin-AKI.

Résumé

Le cisplatine, un agent chimiothérapeutique, s’accumule dans le rein, où il entraîne des lésions rénales aiguës (LRA). Les études précliniques et cliniques laissent entendre que les jeunes souris femelles et les jeunes femmes parviendraient mieux à se rétablir de la situation cisplatine-LRA que les jeunes mâles et les jeunes hommes. L’endothéline (ET) et les récepteurs de l’ET sont plus présents dans les reins et pourraient être dysfonctionnels en situation cisplatine-LRA; cependant

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Author contributions

KAH and SP designed and conducted the experiment. KAH, SP, and AG analyzed the samples and compiled the figures. KAH and AG wrote the manuscript. KAH, SP, and AG edited and approved the manuscript.

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Competing interests

Kelly A.Hyndman is a guest editor of the ET17 Conference Proceedings in CJPP but was not involved in any way in the review process of this manuscript. There are no other conflicts of interest to disclose.

Supplementary material

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nos connaissances quant aux effets éventuels du sexe et du cisplatine dans le système ET rénal sont lacunaires. Nous avons formulé une hypothèse selon laquelle l'expression du système ET rénal est accrue chez les souris mâles et femelles cisplatine-LRA. Comme prévu, les souris cisplatine-LRA présentaient toutes des lésions rénales et une perte de poids plus importantes que les souris témoin. Les souris cisplatine-LRA exprimaient *Edn1*, *Edn3*, *Ednra*, et *Ednrb* dans le cortex dans une plus grande mesure, tandis qu'*Ednra* était nettement inhibé chez les deux sexes. Parmi environ 25 000 gènes séquencés dans la médulla interne, uniquement 91 gènes (comparaison avec les souris solution saline) et 134 gènes (comparaison avec les souris cisplatine-LRA) s'exprimaient de manière différentielle, et ils n'étaient pas liés au système ET. Cependant, *Edn1* s'exprimait nettement plus dans la médulla interne des souris cisplatine-LRA mâles et femelles. Par conséquent, les profils du système ET en ARN étaient affectés de façon marquée avec le couple cisplatine-LRA à l'échelle du rein entier, sans égard au sexe. Cette observation pourrait contribuer à établir le potentiel thérapeutique du ciblage des récepteurs de l'ET en situation cisplatine-LRA.

Keywords

endothelin; kidney; RNA; cisplatin; acute kidney injury; sex

Mots-clés :

endothéline; reins; ARN; cisplatine; lésions rénales aiguës; sexe

Introduction

The endothelin (ET) system is composed of three different peptides ET-1 (gene *Edn1*), ET-2 (*Edn2*), and ET-3 (*Edn3*) that undergo proteolytic cleavage by the ET converting enzymes (*Ece1* and *Ece2*) to form the 21 amino acid active peptides (Davenport et al. 2016). The ETs bind to two different G-protein-coupled receptors, ET_A (*Ednra*) and ET_B (*Ednrb*) (Davenport et al. 2016). Although the endothelium produces ET-1 (Yanagisawa et al. 1988), the inner medulla (IM) of the kidney is a rich source of ET (Kitamura et al. 1989; Morita et al. 1991). Both ET-1 and ET-3 are produced in rodents by the IM (Kitamura et al. 1989), with greatest expression by the IM collecting duct (IMCD) (Kohan 1991). ET-1 is the predominant isoform produced by the IM in humans (Morita et al. 1991). The renal ET system plays a critical physiological role in fluid–electrolyte balance by promoting sodium excretion (Kohan 2011) and dysfunctional renal ET signaling can lead to vasoconstriction, fibrosis, and (or) inflammation (Dhaun et al. 2012). The ET system is therefore hypothesized to promote the pathogenesis of different renal diseases including acute kidney injury (AKI) (Lopez-Farre et al. 1991; Kohan 1994), chronic kidney disease (CKD) (Dhaun et al. 2012, 2013), diabetic kidney disease (Heerspink et al. 2019, 2021a, 2021b; Waijer et al. 2021), and sickle cell nephropathy (Heimlich et al. 2016; Kasztan et al. 2017).

AKI is an abrupt decrease in kidney function that is associated with many complications and can lead to CKD, end-stage kidney disease, other organ dysfunction, or death (Kellum et al. 2021). There are many factors that can lead to the onset of AKI including the use of

nephrotoxic drugs such as cisplatin (*cis*-diamminedichloroplatinum(II)), a chemotherapeutic drug used to treat a large number of cancers (McSweeney et al. 2021). Cisplatin accumulates in the proximal tubules of the kidneys leading to acute tubular necrosis, alters hemodynamics, and can evoke an inflammatory response (McSweeney et al. 2021). Despite adequate hydration and dosing adjustments used in the clinic, nephrotoxicity is still a major concern (Perazella et al. 2022) especially in patients with preexisting conditions (Duan et al. 2020). Biological sex may be an important risk factor for cisplatin-AKI. Older women (Chen et al. 2017) and older female mice in preclinical studies (Boddu et al. 2017) have a higher risk of developing cisplatin-AKI than men or male mice. Thus, there is a need to understand sex-specific signaling and mechanisms, especially in the kidney, to help prevent cisplatin-AKI.

There is existing evidence suggesting that sex differences exist in the renal ET system (Gohar et al. 2016), but data are limited on whether these differences are maintained with cisplatin-AKI. Previous studies have determined that male mice had an increase in whole kidney *Edn1* without a change in *Ednra* or *Ednrb* expression 3 days after a cisplatin-AKI (Lee and Ahn 2008). In male rats with cisplatin-AKI, co-treatment with the ET_A antagonist, BQ-123, attenuated kidney damage (Helmy et al. 2014; Abdel Moneim et al. 2019); however, the dual ET receptor antagonist, bosentan, did not prevent cisplatin-AKI in either male or female rats (Jokar et al. 2015). It is still unclear whether there are sex-specific differences in the kidney ET system in response to cisplatin-AKI. The aim of this study was to determine the expression of the ET system in both male and female mice at baseline and following cisplatin-AKI. We hypothesized that male and female mice have an increase in the expression of the kidney ET system following cisplatin-AKI.

Materials and methods

Animals and tissue collection

All animal use and welfare adhered to the NIH *Guide for the Care and Use of Laboratory Animals* following a protocol reviewed and approved by the Institutional Laboratory Animal Care and Use Committee of the University of Alabama at Birmingham (UAB). Male and female C57bl/6 J mice at 8 weeks of age were purchased from Jackson Labs (Bar Harbor, ME). Mice were acclimated to the UAB animal facility in a room with a 12 h light:12 h dark cycle and fed an ad libitum standard chow diet (0.49% NaCl Teklad #96208) and autoclaved tap water. At 9 weeks of age, each cage of mice was randomly assigned to either saline (vehicle control) or cisplatin. Between 7 and 8 am, when the animal room lights switched on, each mouse received a single, intraperitoneal injection of 100 μL of saline or 15 mg/kg cisplatin (Sigma, St. Louis, MO) dissolved in saline. This dose of cisplatin was used because both male and female mice survive at least a week, while a 20 mg/kg dose led to significant mortality between 48 and 72 h in our lab. At 72 h after the 15 mg/kg injection, the mice were anesthetized with 2.5% vaporized isoflurane, and terminal tissues and blood were collected by a person blinded to the groups.

Blood was collected into a heparinized syringe via cardiac puncture, and plasma was isolated following centrifugation at 4 °C, 1000 *g* for 10 min. Plasma was used to measure creatinine at the UAB-UCSD O'Brien Bioanalytical core using liquid chromatography

coupled to tandem mass spectrometry. Plasma urea concentration was measured using colorimetric assay (BioAssay Systems, QuantiChrom DIUR-100).

Kidneys were excised and decapsulated, and the right kidney was dissected into cortex, outer medulla (OM), and IM. These samples were immediately snap frozen with liquid nitrogen and stored at −80 °C. The left kidney was cut into cross-sections and placed in 10% neutral buffered formalin for 24 h at room temperature (25°C) and stored in 70% ethanol in water until embedded in paraffin for histological analyses.

Histology

The kidney cross-sections were processed and embedded in paraffin with the UAB Animal Resource Program histopathology core. These kidney blocks were then cut at 4 μm sections and placed on superfrost plus slides (Fisher Scientific). Slides were then stained with Gomori's trichrome (Epredia, Richard Allen, Kalamazoo, MI), dehydrated, cleared with Xylenes, and mounted with Cytoseal 60 and a glass coverslip. A researcher blinded to the groups took images of each sample and assessed tubular injury by noting the presence of protein casts or dilated tubules. Representative images reported in this study were taken with an Olympus Bx53 and DP28 digital camera.

RNA isolation, cDNA, and real-time quantitative PCR

RNA was isolated from cortex and OM samples, using the Zymo Quick-RNA miniprep kit. Quantity was determined by the nanodrop and 1 μg of total RNA reversed transcribed with the VILO Superscript IV (Thermo Fisher Scientific). All cDNA was diluted 1/10 with sterile, RNAse-, DNase-free water. Relative transcript expression was determined with SYBR green-based real-time quantitative PCR (qPCR). Each sample was run in duplicate with SSoAdvanced Universal SYBR green master mix (Bio-Rad, Carlsbad, CA), 7.5 pmol of each primer (Table 1), and 1 μL of 1/10 cDNA. Cycle thresholds were determined using the CXF96 system (Bio-Rad).

RNA sequencing of the inner medulla

Because the ET system is most highly expressed in the IMCD (Kitamura et al. 1989; Kohan 1991; Morita et al. 1991), we performed unbiased RNA sequencing of the IM. Total RNA was extracted as mentioned above and sent to GeneWiz (South Plainfield, NJ) where RNA quality was confirmed with Qubit assay and quantitated by qPCR. All samples had an RNA integrity number value >8 except for male cisplatin sample 3 that was 7.9. cDNA libraries were generated and included poly A selection (Illumina, San Diego, CA), and they were sequenced on the Illumina HiSeq3000/4000 in a 2 × 50 base pair configuration targeting 30 million reads per sample. Raw fastq files were analyzed at UAB and have been deposited into Gene Expression Omnibus (GSE195786). The fastq file quality was checked with FASTQC in the package Trim_Galore! and adapter sequences trimmed. Again, file quality was checked with FASTQC, and the files were then mapped to the mouse genome (mm10) with the program STAR. The BAM files generated were then used to determine raw counts with featureCounts in the package subread. Finally, these count matrixes were used in the R package, DESeq2, to determine differentially expressed genes (DEGs). To determine pathways that may be enriched in either sex, the DEG (adjusted p value <0.05) gene symbols

were queried in the DAVID Bioinformatics Resource (v.6.8) and significant (Benjamini p value <0.05) Gene Ontology pathways were determined (Jiao et al. 2012).

Sample sizes, data reporting, and statistics

For sample size determination, we used an a priori alpha = 0.05, with a power of 80%, to detect a 20% difference in mean value and assuming a variation of 10% for a two-mean comparison of a continuous variable. A sample size of four per group was calculated. We collected samples from three cohorts of mice over a 1-year period that resulted in a final sample size reported in Table 2. For qPCR analyses, we extracted RNA from the cortex of $n = 5–7$ mice and RNA from the OM of $n = 5$/group. For qPCR data, all genes of interest were standardized to the housekeeping gene, *Gapdh*, and normalized to the male saline group using the $2^{\Delta\Delta CT}$ method. Statistical significance was determined by a two-factor analysis of variance (ANOVA) (sex and cisplatin) and Tukey's post hoc analysis. $p < 0.05$ was considered statistically significant and group effects and post hoc test p values are reported in the figures. All data are reported as mean ± standard error of the mean. For the RNA sequencing, we extracted RNA from all mice in the study and pairwise comparisons were made between (*i*) male saline and female saline to test for sex differences; (*ii*) male saline and cisplatin; or (*iii*) female saline and cisplatin to test for the effects of cisplatin; or (*iv*) female cisplatin and male cisplatin to test for the effects of sex on cisplatin-related DEGs. Normalized counts from these pairwise comparisons are reported and defined as the raw counts divided by the median ratio of gene counts relative to the geometric mean per gene. Statistical significance was determined by the Wald test with Benjamini and Hochberg correction and adjusted p value <0.05 were considered significant. Volcano plots of the DEG between males and females represent the $\log_2$ fold change versus the −log adjusted p value.

Results

Cisplatin-induced kidney injury

Three days after cisplatin or vehicle control injection, cisplatin-treated male and female mice had a significantly higher plasma creatinine ($P_{sex} = 0.19$, $P_{cisplatin} = 0.019$, and $P_{Interaction} = 0.11$) and plasma urea ($P_{sex} = 0.072$, and $P_{cisplatin} = 0.014$, and $P_{Interaction} = 0.43$) compared to vehicle-injected mice (Table 2). Moreover, cisplatin-injected mice had a significant 13%–14% loss in body mass, while there was no significant change in body mass of control mice (Table 2). Kidney and heart masses were smaller in the female mice compared to male mice, regardless of cisplatin treatment; however, when corrected for starting body mass, cisplatin-treated mice had significantly lower kidney/body mass ($P_{sex} = 0.85$, $P_{cisplatin} = 0.006$, and $P_{Interaction} = 0.3$) and lower heart/body mass ($P_{sex} = 0.20$, $P_{cisplatin} = 0.016$, and $P_{Interaction} = 0.95$) (Table 2).

The kidney structure was normal in the male and female control mice with no protein casts or dilated tubules observed (Fig. 1). In contrast, cisplatin-treated mice had multiple protein casts in the cortex, OM and IM, and notable dilated tubules (Fig. 1). Thus, our dosing of 15 mg/kg of cisplatin produced kidney damage in all of the cisplatin-treated mice, regardless of sex.

Cortical kidney endothelin system expression in males and females

Next, the mRNA expression of the ET system was determined in the cortex, OM, and IM. In the cortex, cisplatin led to a threefold increase in *Edn1* regardless of sex (P_{sex} = 0.87, $P_{cisplatin}$ = 0.0047, and $P_{Interaction}$ = 0.93; Fig. 2A). The kidneys also expressed *Edn3*, and this was 50% greater in the cisplatin-treated mice, regardless of sex (P_{sex} = 0.89, $P_{cisplatin}$ 0.0072, and $P_{Interaction}$ = 0.44; Fig. 2B). We were unable to detect *Edn2* in the cortex. There were no statistically significant differences in cortical *Edn1* or *Edn3* expression between the sexes (Figs. 2A and 2B). The ET converting enzyme-1 (*Ece1*) was expressed in the cortex; however, its expression was not significantly affected by sex or cisplatin treatment (P_{sex} = 0.39, $P_{cisplatin}$ = 0.61, and $P_{Interaction}$ = 0.66; Fig. 2C). *Ece2* was not detected in the cortex. Both *Ednra* and *Ednrb* were expressed in the cortex and were expressed ~50% more in cisplatin-treated mice (Figs. 2D and 2E). Thus, the cisplatin-treated mice had generally increased transcript abundance of the cortical ET system.

Outer medullary kidney endothelin system expression in males and females

Unlike the cortex, the OM ET system had few significant sex or cisplatin-induced differences. *Edn1* (P_{sex} = 0.76, $P_{cisplatin}$ = 0.17, and $P_{Interaction}$ = 0.23; Fig. 3A) and *Edn3* (P_{sex} = 0.16, $P_{cisplatin}$ = 0.60, and $P_{Interaction}$ = 0.47; Fig. 3B) were not significantly different between the sexes or with cisplatin treatment. *Edn2* was not detected in the OM. Likewise, *Ece1* was not significantly different among the groups (P_{sex} = 0.75, $P_{cisplatin}$ = 0.21, and $P_{Interaction}$ = 0.25) and *Ece2* was not detected (Fig. 3C). In the OM, *Ednra* was ~25% less expressed in the cisplatin-treated mice than controls in both sexes (P_{sex} = 0.88, $P_{cisplatin}$ = 0.022, and $P_{Interaction}$ 0.88; Fig. 3D).The *Ednrb* was expressed ~50% greater in female mice compared to males, regardless of cisplatin (P_{sex} = 0.045, $P_{cisplatin}$ = 0.53, and $P_{Interaction}$ = 0.75; Fig. 3E).

Inner medullary RNA sequencing from male and female mice

Our lab has a standing interest in the molecular mechanisms in the IM, and given that the IMCD produces the most ET-1 in the kidney (Kohan 1991), we performed unbiased RNA-sequencing on the IM. There were more than 25 800 genes sequenced from the IM, but when comparing male and female control mice in the IM, there were only 91 DEGs (adjusted *p* value <0.05) (Fig. 4A, Table S1, https://doi.org/10.6084/m9.figshare.19222659.v1). Enriched in the male IM were the *Slc22a* genes (Fig. 4A), which are part of the drug transporter family including the organic anion transporters (OATs) and organic cation transporters (OCTs) (Nigam 2018). Also, not surprisingly, there were Y chromosome-linked genes expressed in the male mice (*Uty*, *Ddx3y*, *Kdm5d*, and *Eif2s3y*). In the female control mice, there was significantly greater expression of the gene *Xist* (involved in X-inactivation), the amino acid transporter, *Scl7a12*, and renin (*Ren1*) (Fig. 4A). Gene Ontology analyses with these 91 genes highlighted that there are significant sex differences in biological pathways such as sodium-independent organic anion transport (fold enrichment = 61.9, number of genes = 7, and Benjamini *p* value = 4.6E–7), and oxidation–reduction process (fold enrichment = 4.8, number of genes = 13, and Benjamini *p* value = 0.0027).

When we compared normalized counts of ET-related transcripts, there were no statistically significant differences in expression of *Edn1*, *Edn2*, *Ece1*, *Ednra*, or *Ednrb* between control male and female mice in the IM (adjusted p value <0.05, Figs. 4B–G). Similar to the cortex and OM, *Edn2* was not detected/sequenced, and *Ece2* had low normalized counts (Fig. 4E).

In females, cisplatin-treated mice had twice the IM *Edn1* expression than control female mice ($p = 0.0009$; Fig. 5A). However, there were no statistically significant differences in female IM *Edn2*, *Ece1*, *Ednra*, or *Ednrb* between cisplatin- and saline-treated mice (Figs. 5B–F). There was a small but statistically significant greater expression of *Ece2* in cisplatin-treated female mice (Fig. 5D and Table S1). In males, we likewise detected significantly more *Edn1* transcripts in cisplatin-treated mice compared to control ($p = 2.03E–8$; Fig. 6A) but no statistical differences in the normalized counts of *Edn3*, *Ece1*, or *Ece2* (Figs. 6B–D). Both *Ednra* and *Ednrb* transcripts were significantly fewer in the cisplatin-treated male mice (Figs. 6E and 6F; Table S1).

Finally, we compared cisplatin-treated male and cisplatin-treated female mice. Although there were 134 DEGs between these mice (Fig. 7A; Table S1), 53 genes lacked official gene symbols (they were gm or rik genes). Thus, although there were sex-specific genes differentially regulated in cisplatin-treated mice, many were novel genes whose function still requires determination. Of the 134 DEGs, 25 are sexually dimorphic as they were also found in the male versus female saline groups (Fig. 4A, e.g., *Xist* and *Uty*). Moreover, Gene Ontology analyses failed to detect any statistically significant pathways enriched by these 134 genes. Finally, there were no statistically significant differences between the ET systems of cisplatin-treated female or male mice (Figs. 7B–G).

Discussion

Although the incidence of cisplatin-AKI has improved with therapeutic approaches such as forced hydration and lower dosing, cisplatin-AKI still affects a significant portion of cancer patients (Perazella et al. 2022). Thus, there is a need to better understand the mechanisms related to cisplatin-AKI to determine alternative therapeutic strategies. One such avenue may be targeting the renal ET system. The Study of Diabetic Nephropathy with atrasentan (SONAR, NCT01858532) was a double-blind, randomized, placebo-controlled large trial that determined that ET_A blockade reduced the risk of kidney events in diabetic and CKD (Heerspink et al. 2019, 2021b). This clinical trial solidified the importance of the ET system in kidney health and has led to new hypotheses about how the renal ET system may be affected in disease states, making it a desirable therapeutic target. Thus, the goal of the current study was to determine whether cisplatin-AKI affects the kidney ET system in a preclinical model of both sexes.

As expected, cisplatin resulted in significant kidney injury (e.g., dilated tubules and protein casts) to both young adult male and female mice in this study. Moreover, all cisplatin-AKI mice had a significant reduction in body mass that was associated with a significant increase in plasma urea regardless of sex. This is consistent with the dehydration associated with the cisplatin model (Perse 2021). Biological sex (Chen et al. 2017) and age (Latcha et al. 2016) are important variables that affect kidney outcomes following AKI and generally younger

females having some protection from reduced renal function associated with cisplatin-AKI even though they may have kidney injury (Boddu et al. 2017; Hwang et al. 2021). Thus, the phenotype of our cisplatin-AKI model matches well with other preclinical models.

Shortly after the discovery of ET (Yanagisawa et al. 1988), it became apparent that the ET system is heterogeneously expressed throughout the kidney. ET receptors are enriched in areas including the glomerulus, vasculature, interstitial cells, and IMCD (Kohzuki et al. 1989; Yukimura et al. 1996). Unlike previous studies that determined ET system expression in whole kidney homogenates from rodent models with or without cisplatin-AKI (Lee and Ahn 2008; Hwang et al. 2021), we separated the cortex, OM, and IM for our RNA analyses. Making these determinations is important for understanding whether endothelin receptor antagonists (ERAs) will have therapeutic potential in cisplatin-AKI or other kidney disease models. In the cortex, cisplatin-AKI mice had greater *Edn1*, *Edn3*, *Ednra*, and *Ednrb* expression with no statistically significant sex differences detected. In the OM, cisplatin-AKI mice had significantly reduced *Ednra*, and female mice had significantly greater *Ednrb* expression than male mice regardless of cisplatin treatment. Finally, in the IM, all cisplatin-AKI mice had significantly higher *Edn1* while only in males did cisplatin-AKI result in less *Ednra* and *Ednrb* expression. These findings expand upon previous studies that determined whole kidney *Edn1* was increased following cisplatin-AKI in male mice; however, whole kidney *Ednra* and *Ednrb* were not statistically different between the groups (Lee and Ahn 2008). Thus, our study provides strong evidence that ET system is differentially affected cisplatin-AKI throughout the whole kidney. For example, in a male rat model of cisplatin-AKI, the ET_A receptor antagonist, BQ-123, when administered at the same time as the cisplatin, provided some kidney protection (serum creatinine, urea, and tubular necrosis were improved) compared to control cisplatin rats (Abdel Moneim et al. 2019). However, the dual receptor antagonist, bosentan, did not provide any protection from the cisplatin-induced rise in plasma creatinine or blood urea nitrogen in male (Helmy et al. 2014; Jokar et al. 2015) or female rats (Jokar et al. 2015). These studies suggest that in cisplatin-AKI, it is the ET_A receptors and perhaps those that are increased in the cortex that are dysfunctional and contribute to a loss of kidney function. Similar findings have been reported in sickle cell disease nephropathy (Kasztan et al. 2017) and diabetic nephropathy (Saleh et al. 2011; Spires et al. 2018; Heerspink et al. 2019).

Although we did not detect a significant effect of sex on the susceptibility to cisplatin-AKI (no statistical differences in plasma creatinine or urea, and there was evidence of kidney injury in all cisplatin-treated mice), we did find sex-specific differences in the ET system and other genes in our IM transcriptome. Previous studies have clearly demonstrated that although kidney function is similar between the sexes, the mechanisms that are used to maintain homeostasis are distinct (Veiras et al. 2017; Hu et al. 2019, 2020, 2021; Torres-Pinzon et al. 2021). This also may relate to differences in the kidney ET system (Gohar et al. 2016) and susceptibility to cisplatin-AKI (Boddu et al. 2017; Chen et al. 2017; Hwang et al. 2021). Gohar et al. (2022) reported that the expression of *Ece2*, *Ednra*, and *Ednrb* was greater in the IM of healthy female versus male rats. We found no statistically significant sex differences in the IM or cortex mRNA expression of ET systems in healthy mice or cisplatin-AKI mice. Similarly, Hwang et al. (2021) published their kidney transcriptome data from healthy or cisplatin-AKI male and female mice, and in agreement with our study,

did not detect a significant difference (>2-fold change, $p < 0.05$, false discovery rate < 0.01) in the kidney ET system. This suggests that species-specific differences may exist. Unfortunately, studies using human kidney biopsies have been underpowered to test for sex-specific expression or protein abundance (Pupilli et al. 1994; Karet and Davenport 1996; Frank et al. 2006). Healthy women do excrete more ET-1 than healthy, age-matched males (Gohar et al. 2022), and urinary ET-1 excretion likely reflects kidney ET-1 production (Dhaun et al. 2009). Studies rigorously testing potential sex differences in the ET system in the human kidney are warranted.

We found in the IM that multiple *Slc22a* genes were expressed higher in saline male mice compared to saline female mice. This is the gene family that includes the OATs and OCTs (Nigam 2018). It is well established that there is high expression of OATs/OCTs in the proximal tubules of preclinical models and the human kidney (Saito 2010), and they function in tubular secretion of metabolites, xenobiotics, and drugs like cisplatin (Sekine et al. 2006). Recent single cell/nucleus RNA sequencing has also confirmed that *Slc22a* genes are expressed in other epithelial cells like the principal cell (Hyndman et al. 2020; Hyndman and Crossman 2022). *Slc22a6* (OAT1), for example, was 2.9-fold higher in the male IM than female (adjusted p value = 0.003), and others have also reported that females have only 40% of the *Slc22a6* expression of males in the kidney (Cerrutti et al. 2002). Other *Slc22a* genes have greater expression in the male kidney as well and this may help explain why males (Boddu et al. 2017; Hwang et al. 2021) and men (Latcha et al. 2016) generally have more severe cisplatin-AKI. In our cisplatin male versus female comparison, there were an additional 109 genes differentially expressed (25 overlapped with the saline male and female dataset) and majority were genes whose functions are unknown and are likely unrelated to the ET system.

There are limitations to our study. We reported relative RNA expression, so caution needs to be taken when extrapolating to protein expression or receptor activation. Unfortunately, validated and commercially available antibodies for ET_A or ET_B are not available. This is a particular problem for studies investigating these and many other G-protein-coupled receptors (Herrera et al. 2013; Baker 2015; Chappell 2016). Often, changes in the mRNA expression of the ET system do correlate and agree with the findings of ERA interventions (e.g., Kasztan et al. 2017; Abdel Moneim et al. 2019). Our design was also completed soon after a single dose of cisplatin, and, therefore, our results may not reflect long-term changes in the kidney or correlate with different cisplatin dosing regimens.

These preclinical findings lead us to conclude that the kidney ET system is differentially affected following cisplatin-AKI and that sex-specific gene differences unrelated to the ET system may explain susceptibility and incidence of cisplatin-AKI in the mouse model. Future studies are needed to determine if there are sex-specific outcomes to ERAs and whether targeting the kidney ET system can prevent cisplatin-induced nephrotoxicity leading to AKI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

The RNA sequencing data are freely available from GEO as described in the “Materials and methods” section. All supplementary tables are available at figshare: https://doi.org/10.6084/m9.figshare.19222659.

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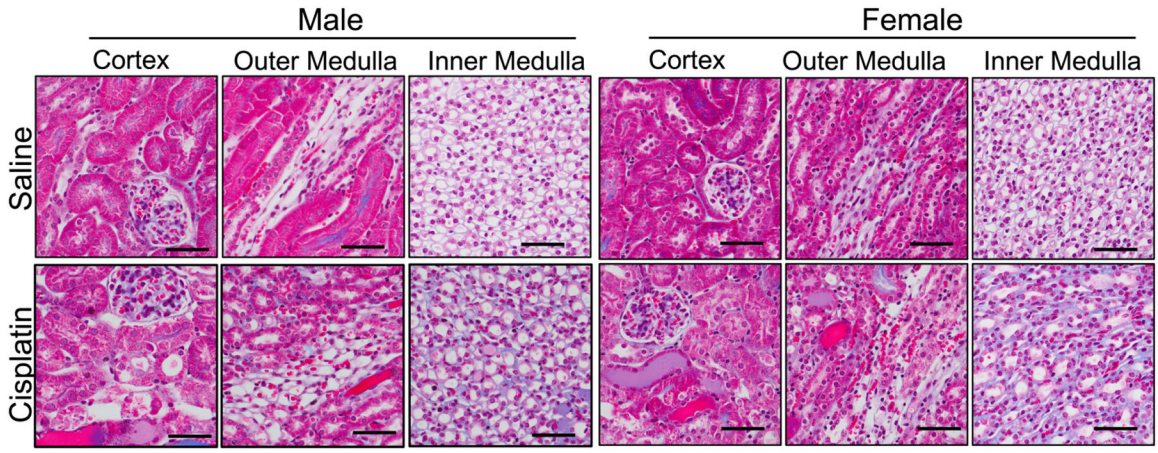

Fig. 1.

Representative trichrome stained kidney sections from mice that received a single intraperitoneal injection of either saline (vehicle control) or 15 mg/kg of cisplatin 3 days prior. Scale bar is 50 μm.

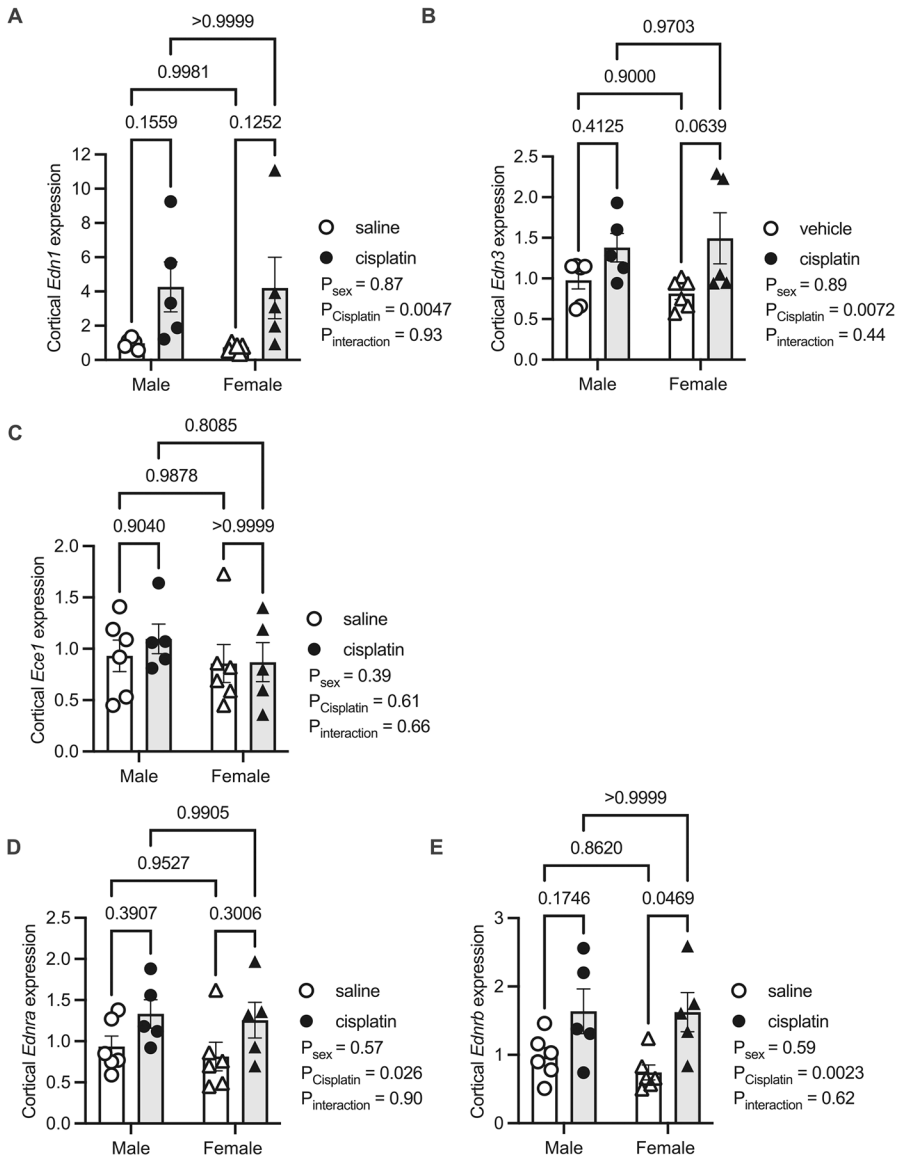

Fig. 2.

Kidney cortex samples from male and female mice 3 days following a single intraperitoneal injection of saline (vehicle) or 15 mg/kg cisplatin. Real-time quantitative PCR of various endothelin (*Edn*) related genes from these cortex samples. (A) *Edn1*, (B) *Edn3*, (C) *Ece1*, (D) *Ednra*, and (E) *Ednrb*. All values are relative to the male saline group. *P* values from a two-factor ANOVA reported and results of Tukey's post hoc are represented by lines with the *p* value reported. Sample sizes: male saline = 6, male cisplatin = 6, female saline *n* = 6, and female cisplatin = 5.

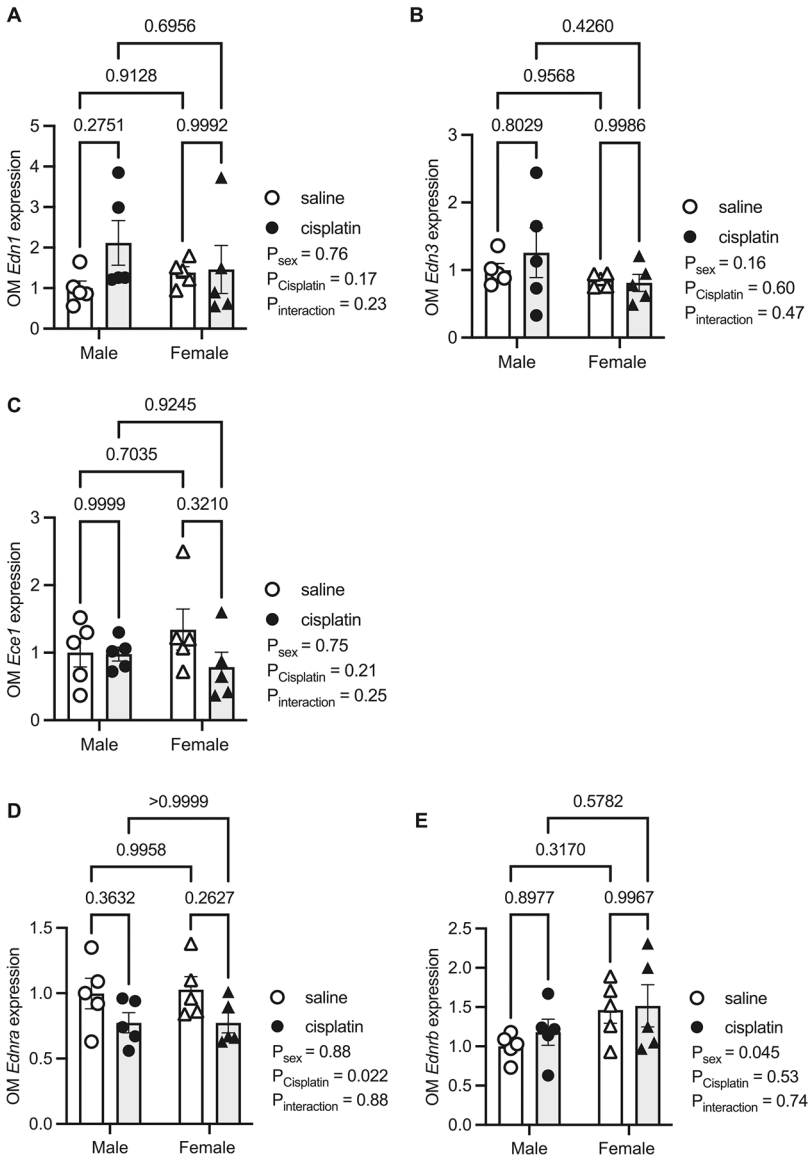

Fig. 3.

Kidney outer medulla (OM) samples from male and female mice 3 days following a single intraperitoneal injection of saline (vehicle) or 15 mg/kg cisplatin. Real-time quantitative PCR of various endothelin (*Edn*) related genes from these cortex samples. (A) *Edn1*, (B) *Edn3*, (C) *Ece1*, (D) *Ednra*, and (E) *Ednrb*. All values are relative to the male saline group. *P* values from a two-factor ANOVA reported and results of Tukey's post hoc are represented by lines with the *p* value reported. $N = 5$ per group/sex.

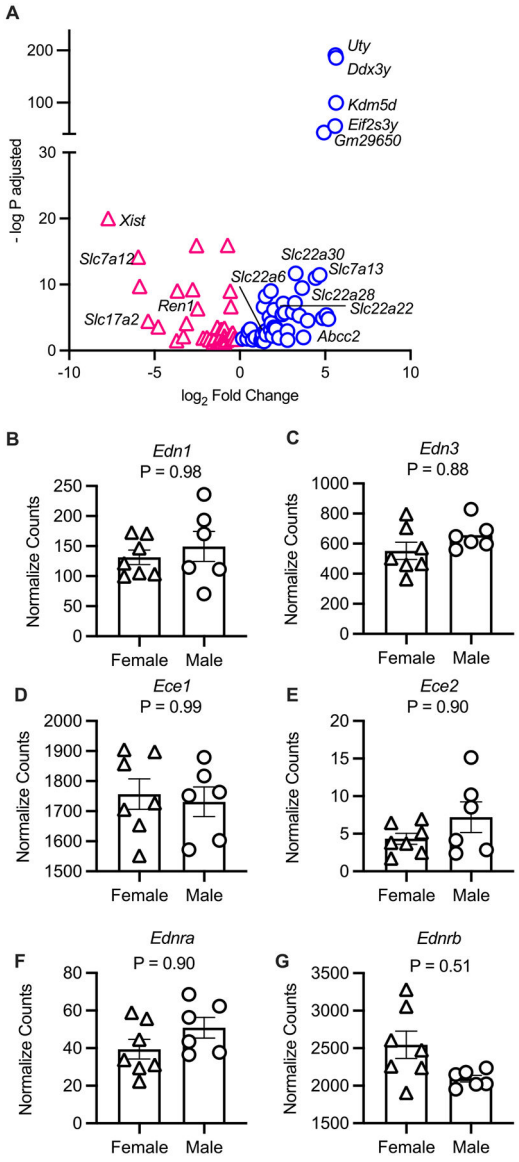

Fig. 4.

Differentially expressed genes in the inner medulla of male and female saline-injected mice. (**A)** A volcano plot of the significant (adjusted *p* value <0.05) differentially expressed genes in males (blue) compared to females (pink). (B–G) Comparisons of the inner medullary endothelin (*Edn*) related transcripts from female and male saline-injected mice from this study. Normalized counts from the analyses package DESeq2 are reported and *p* values are adjusted *p* values from the Wald test with Benjamini and Hochberg correction reported. (B) *Edn1*, (C) *Edn3*, (D) *Ece1*, (E) *Ece2*, (F) *Ednra*, and (G) *Ednrb*. Sample size: females = 7 and males = 6.

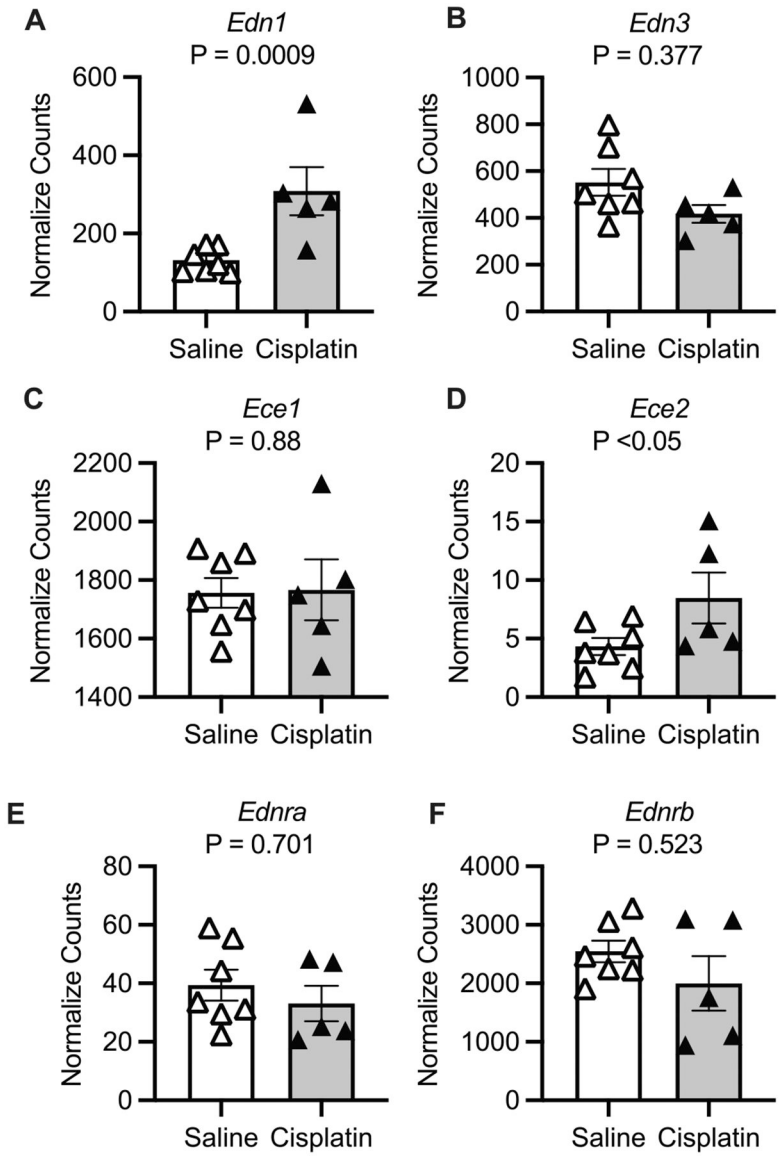

Fig. 5.

Kidney inner medulla samples from female mice 3 days following a single intraperitoneal injection of saline (vehicle) or 15 mg/kg cisplatin. Normalized counts from the analyses package DESeq2 are reported and *p* values are adjusted *p* values from the Wald test with Benjamini and Hochberg correction reported. (A) *Edn1*, (B) *Edn3*, (C) *Ece1*, (D) *Ece2*, (E) *Ednra*, and (F) *Ednrb*. Sample size: saline = 7 and cisplatin = 5.

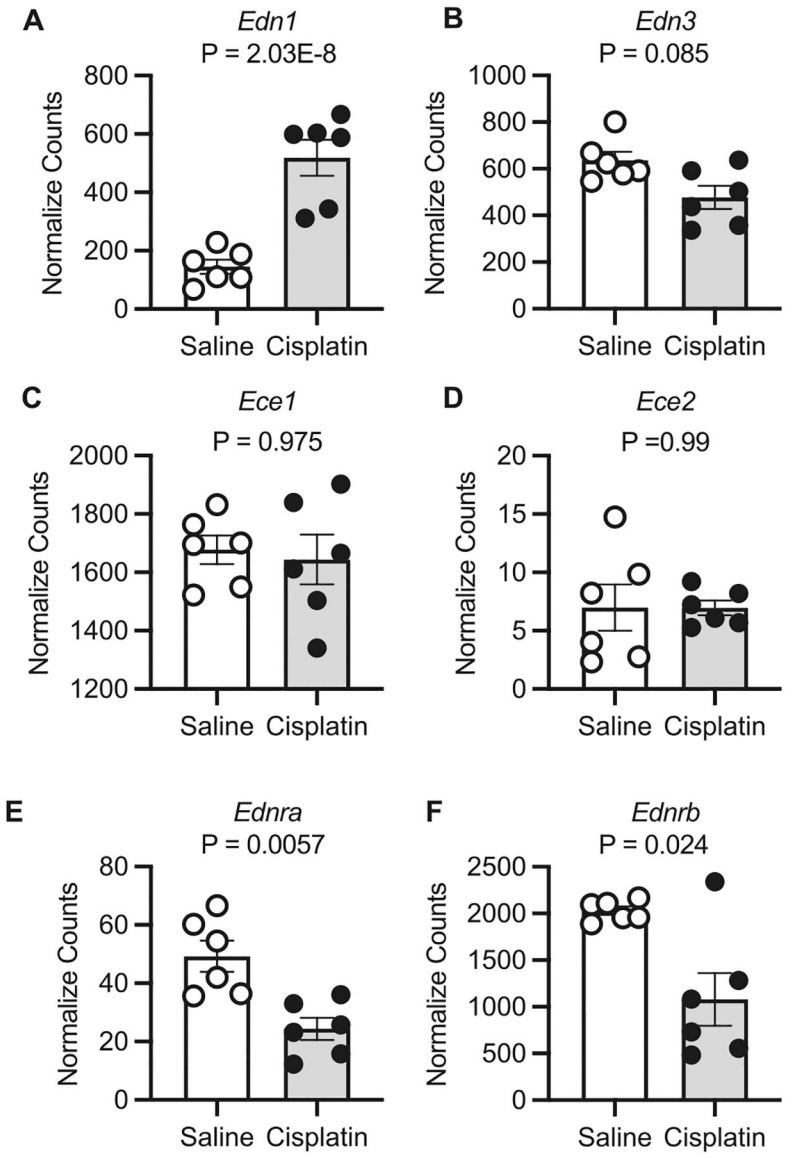

Fig. 6.

Kidney inner medulla samples from male mice 3 days following a single intraperitoneal injection of saline (vehicle) or 15 mg/kg cisplatin. Normalized counts from the analyses package DESeq2 are reported and *p* values are adjusted *p* values from the Wald test with Benjamini and Hochberg correction reported. (A) *Edn1*, (B) *Edn3*, (C) *Ece1*, (D) *Ece2*, (E) *Ednra*, and (F) *Ednrb*. Sample size = 6/group.

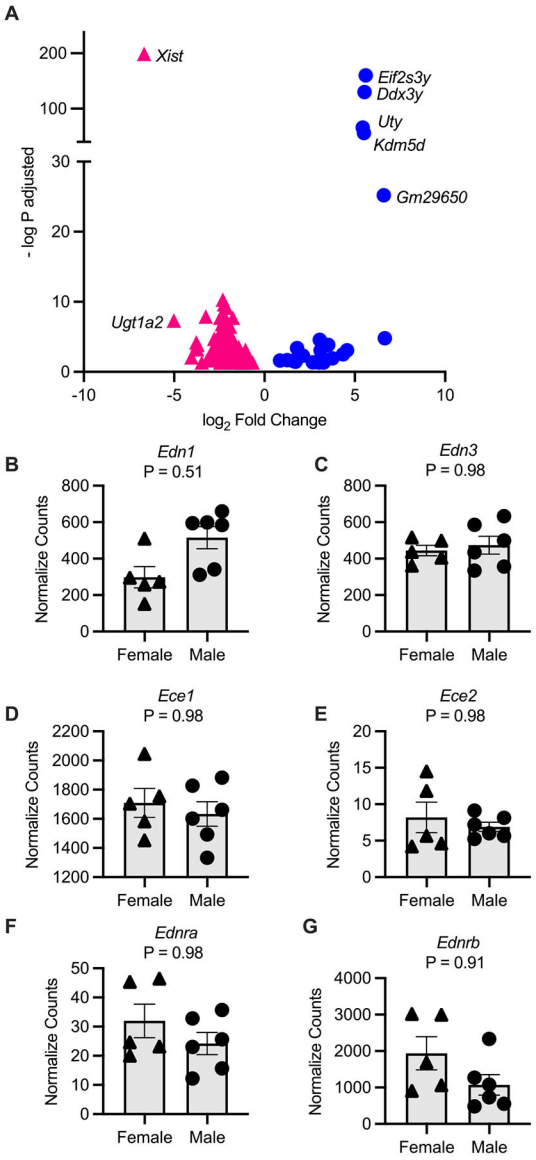

Fig. 7.

Differentially expressed genes in the inner medulla of male and female cisplatin-injected mice. (A) A volcano plot of the significant (adjusted *p* value <0.05) differentially expressed genes in males (blue) compared to females (pink). (B–G) Comparisons of the inner medullary endothelin (*Edn*) related transcripts from female and male cisplatin-injected mice from this study. Normalized counts from the analyses package DESeq2 are reported and *p* values are adjusted *p* values from the Wald test with Benjamini and Hochberg correction reported. (B) *Edn1*, (C) *Edn3*, (D) *Ece1*, (E) *Ece2*, (F) *Ednra*, and (G) *Ednrb*. Sample size: females = 5 and males = 6.

Table 1.

Real-time quantitative PCR primers used in the study.

Transcript	Forward	Reverse	Amplicon
Edn1	GCACCGGAGCTGAGAATGG	GTGGCAGAAGTAGACACACTC	119
Edn2	CACCTGCGTTTTCGTCGATG	CCAGTGTCTTCGATGGCAGAA	219
Edn3	CCCTGGTGAGAGGATTGTGTC	CCTTGTCCTTGTAAGTGAAGCAC	161
Ece1	TCTCCGAGGGCGATGTGTA	CTTCTCCACCGAGGTCCGA	98
Ece2	GGTGTTGGGGAAGTGTACTGA	AAGCCAGCAGTCCTTCTCTTT	114
Ednra	ATGAGTATCTTTTGCCTTGCGG	GTCTTCCATGTGGCTGCTTAG	108
Ednrb	GTGGCTTCTTGGGGGTATGG	TCTTAGTGGGTGGCGTCATTA	102
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA	123

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Table 2.

Characteristics of the mice used in the study.

		Female		Male		Two-factor ANOVA		
		Saline	**Cisplatin**	**Saline**	**Cisplatin**	P_{sex}	$P_{cisplatin}$	$P_{Interaction}$
Sample size		7	5	6	6			
Age		9	9	9	9			
Starting mass	g	20.38 (0.32)	20.77 (0.55)	**26.49 (0.69)** *	**27.70 (0.41)** *	**<0.001**	0.13	0.43
Change in mass	%	−0.55 (1.49)	**−13.2 (3.80)** *	0.56 (0.92)	**−14.33 (2.78)** *	0.99	**<0.001**	0.62
Total kidney mass	mg	0.24 (0.004)	0.21 (0.004)	0.30 (0.01)	0.29 (0.006)	**<0.001**	0.072	0.38
Kidney/starting body mass	mg/g	11.56 (0.13)	**10.20 (0.21)** *	11.23 (0.36)	10.43 (0.26)	0.85	**0.0006**	0.30
Kidney/dissection body mass	mg/g	11.64 (0.12)	12.03 (0.57)	11.13 (0.33)	**12.12 (0.55)**	0.68	0.069	0.37
Heart mass	mg/dL	0.11 (0.008)	0.092 (0.001)	0.13 (0.007)	0.12 (0.006)	**0.0045**	0.073	0.76
Heart/starting body mass	mg/g	5.10 (0.32)	4.48 (0.14)	4.79 (0.19)	4.20 (0.19)	0.20	**0.016**	0.95
Heart/dissection body mass	mg/g	5.20 (0.34)	5.26 (0.14)	4.78 (0.16)	4.96 (0.17)	0.12	0.60	0.77
Plasma creatinine	mg/dL	0.14 (0.01)	0.16 (0.03)	0.13 (0.007)	**0.25 (0.06)** *	0.19	**0.019**	0.11
Plasma urea	mg/dL	53.2 (6.6)	76.7 (15.6)	66.9 (4.2)	110.7 (20.5)	0.072	**0.014**	0.43

Note: Mean (standard error of mean) values are reported. Bold represents statistical significance.

* indicates significant difference from post hoc Tukey's analysis.

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