

Renal Oxidative Stress and Inflammatory Response in Perinatal Cyclosporine-A Exposed Rat Progeny and its Relation to Gender

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Abstract

Background and Aim of the Work: The current study postulated that cyclosporine A (CSA) could induce gender-specific renal damage. Hence, the current study aims to investigate the nephrotoxic effect of perinatal exposure of male and female rat progeny to CSA. Moreover, it aims to evaluate the oxidative stress and inflammation as a possible pathophysiologic mechanism. **Materials and Methods:** Female rats were randomly allocated to two groups of four and assigned to undergo either CSA (15 mg/kg/day; the 6th day after conception and continuing until the progeny were weaned) or vehicle treatment as control groups. At the age of 6 weeks, the progeny were divided into the following four groups: male progeny of control-group mothers (M-vehicle, 7); male progeny of CSA-treated mothers (M-CSA, 9); female progeny of control-group mothers (F-vehicle, 7); and female progeny of CSA-treated mothers (F-CSA, 6). Serum adiponectin, tumor necrosis factor- α (TNF- α) and creatinine, creatinine clearance, and urinary 8-isoprostane were measured. Histopathological examination by hematoxylin and eosin stain of Kidney was carried out. **Results:** Proteinuria and decreased creatinine clearance are significant in M-CSA than M-vehicle and F-CSA. 8-isoprostane is lower in F-CSA than F-vehicle. Increased TNF- α and decreased adiponectin levels in M-CSA than M-vehicle were observed. No significant differences were found in female rat groups. **Conclusion:** From the current study, it could be concluded that CSA could induce renal inflammation as well as oxidative stress that may explain the impaired renal function. The sex difference was a prominent finding in their vulnerability to CSA effects.

Keywords: Cyclosporine, gender, inflammation, kidney, oxidative stress, perinatal exposure

INTRODUCTION

Cyclosporine A (CSA) is an immunosuppressant drug. It is commonly used in preventing transplanted organ rejection.^[1,2]

Its use usually adversely affects the renal systems. It is reported that CSA-induced nephrotoxicity is characterized by hypertension, lower glomerular filtration rate, higher serum creatinine concentration, and decreased creatinine clearance in both rat models, as well as CSA, treated human patients.^[3-5]

The exact pathophysiological mechanism of CSA-associated nephrotoxicity is not fully understood until now. Many previous studies proposed oxidative stress^[6] and systemic as well as local inflammation as possible underlying mechanisms.^[2,7]

Pharmacokinetics, pharmacodynamics, and toxicodynamics of CSA had been reported to be dependent on both age^[8] and

sex.^[3] Recently, in 2018, Kim *et al.*^[9] reported that male sex is more prone to CSA nephrotoxic effect than the female sex.

The objectives of the present study were designed: First, to study the possible nephrotoxic effect of perinatal exposure of male and female rat progeny to CSA and elucidate the sex difference. Second, to evaluate the oxidative stress and inflammation as a possible pathophysiologic mechanism.

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MATERIALS AND METHODS

Methods

Animals

The present investigation conformed to the Bioethics in Research Regulations implemented by the Kingdom of Saudi Arabia. Male and female Wistar rats that were used for mating were supplied by King Abdulaziz University, the Kingdom of Saudi Arabia. Rats were kept in transparent polypropylene cages with 3–4 rats per cage. The animals had ready access to commercially obtainable rodent food pellets and water and were subjected to constant environmental conditions with equal periods of light and dark.

Experimental design and procedures

In a procedure endorsed by the Ethics Research Committee, King Abdulaziz University, the Kingdom of Saudi Arabia, male and female Wistar rat pairs were placed in individual cages and the date of conception was confirmed by daily vaginal smear testing and weighing. Once pregnant, female rats were randomly allocated to two groups of four and assigned to undergo either CSA or vehicle treatment. CSA group received a dose of 15 mg/kg/day subcutaneously, commencing on the 6th day after conception and continuing until the progeny were weaned.^[10,11] The control group received a vehicle consisting of 18% koliphore and 2% ethanol in sterile saline. At the age of 6 weeks, the progeny was divided into the following four groups: (i) male progeny of control-group mothers (M-vehicle, 7 animals); (ii) male progeny of CSA-treated mothers (M-CSA, 9 animals); (iii) female progeny of control-group mothers (F-vehicle, 7 animals); and (iv) female progeny of CSA-treated mothers (F-CSA, 6 animals).

Then, rats were placed in metabolic cages individually to collect the 24-h urine in order to determine the volume and levels of various parameters such as protein, creatinine and 8-isoprostane, proteinuria, and creatinine clearance. Intraperitoneal injections of ketamine (100 mg/kg) in xylazine (10 mg/kg) were administered to anesthetize the rats before 4 ml of blood were extracted from the vena cava through a small opening in the lower abdomen. This was kept at 4°C for 30 min to coagulate before centrifugation for 20 min at 3,000 g and 4°C. The serum was aspirated, divided into fractions, and stored at –80°C for subsequent tumor necrosis factor- α (TNF- α) and adiponectin analysis. The kidney was fixed in 10% neutral buffered formalin for subsequent histopathological examination by hematoxylin and eosin stain.

Biochemical analyses

ELISA determination was used for the measurements of TNF α (R and D systems[®], Minneapolis, MN, USA), adiponectin, and urine 8-isoprostane (Abcam[®], Cambridge, MA, USA). Serum creatinine and urine creatinine and protein content were quantified by the ELITech[®] assay kits (ELITech, Puteaux, France).

Reagents and drugs

Reagents and drugs used in this study were as follows: cyclosporine (Sandimmune[®], Novartis Pharmaceuticals

Corporation East Hanover, New Jersey), ketamine (Tekam[®], Hikma Pharmaceutical, Amman, Jordan), and xylazine (Seton[®], Laboratories Calier, Barcelona, Spain) (Sigma-Aldrich, St. Louis, MO, USA).

Statistical analysis

Statistical variance analysis (ANOVA) was followed by Newman-Keuls *post hoc* test using statistical software (GraphPad Prism version 5.00 for Windows, GraphPad Software, La Jolla California USA). Values are quoted as the mean \pm standard error of the mean and $P < 0.05$ was considered as statistically significant.

RESULTS

Influence of perinatal cyclosporine A exposure on kidney function

In the control group, proteinuria was significantly higher in female than in male rats. The administration of CSA (15 mg/kg/day s) to pregnant rats, beginning on the 6th day of conception till weaning, led to a sex-linked kidney dysfunction. CSA administration led to a significant increase in proteinuria in male but not female progeny compared with the corresponding control [Figure 1].

In addition, creatinine clearance was significantly lower in female than in male rats. CSA administration led also to a sex-linked decrease in creatinine clearance in male but not female progeny compared with the corresponding control [Figure 2].

Influence of perinatal cyclosporine A exposure on oxidative stress

No sex-related differences were observed for the oxidative stress marker 8-isoprostane in control groups. However, the female rats subjected to perinatal CSA had significantly reduced amounts of 8-isoprostane. 8-isoprostane was not influenced by perinatal CSA exposure in male progeny [Figure 3].

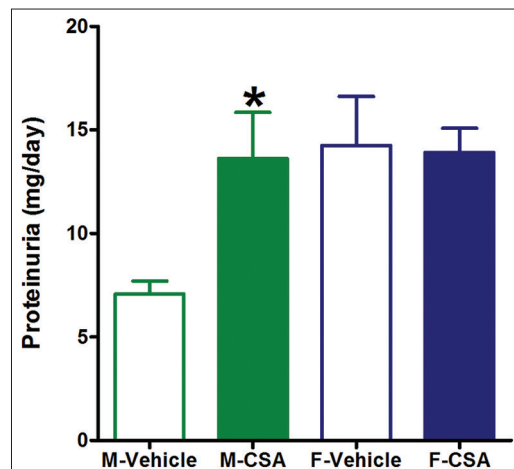


Figure 1: Urinary protein content in the studied rats groups (mg/day)

Influence of perinatal cyclosporine A exposure on inflammatory/oxidative/fibrotic pathways

In the vehicle-exposed rats, ELISA investigations indicated comparable amounts of the anti-inflammatory (adiponectin) and the inflammatory (TNF- α) cytokines in the serum of male and female rats. Perinatal administration of CSA led to significant decreases in serum adiponectin by approximately 40% in the male progeny with no apparent changes in the female progeny [Figure 4]. Further, ELISA studies showed that perinatally CSA-exposed male, but not female, rats exhibited = 30% increase in serum TNF- α level [Figure 5].

Histopathological effects of perinatal cyclosporine A exposure

While no marked difference between kidney cross-sections from male and female progeny, the perinatal exposure to CSA caused vascular congestion and heavy aggregation of chronic inflammatory cells in male but not female progeny [Figure 6].

DISCUSSION

Nephrotoxicity is a well-established common adverse effect of cyclosporine therapy.^[4,12-14] The renal impairments that are

associated with the administration of CSA in experimental rat model are confirmed in the current study in the form of proteinuria, increased serum creatinine level and diminished creatinine clearance. The histopathological examination of the renal tissue proved these biochemical findings. There is evident renal congestion with aggregation of inflammatory cells in the renal tissue. These findings coincide with the previously published results by Lassila *et al.*,^[15] Wu *et al.*,^[16] Lai *et al.*,^[17] and confirmed recently by El-Bassossy and Eid.^[2]

The possible mechanisms by which CSA induces nephrotoxicity can be summarized as follow: direct renal tubular apoptosis^[18] that is mediated by Fas antigen-ligand system, induction of stress response protein glucose transporter 1^[19] and immunoglobulin A nephrotoxicity^[20] with a subsequent development of renal interstitial fibrosis. Renal inflammatory damage could be considered as a strong pathogenic factor of CSA-induced nephrotoxicity.^[21]

In the current study, male rats showed an increased level of urinary 8-isoprostane (oxidative stress marker) than the sham control group. Oxidative stress could be a strong pathogenic factor of

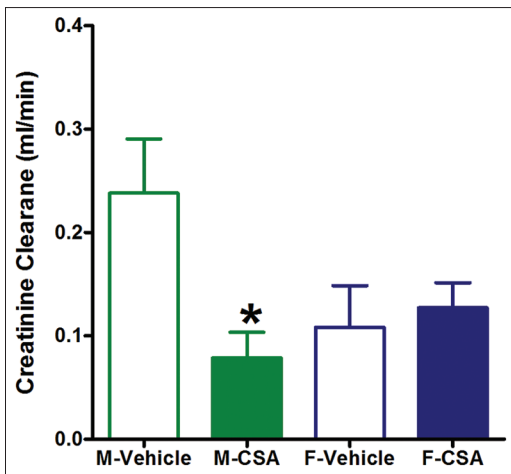


Figure 2: Creatinine clearance of the studied rats groups (ml/min)

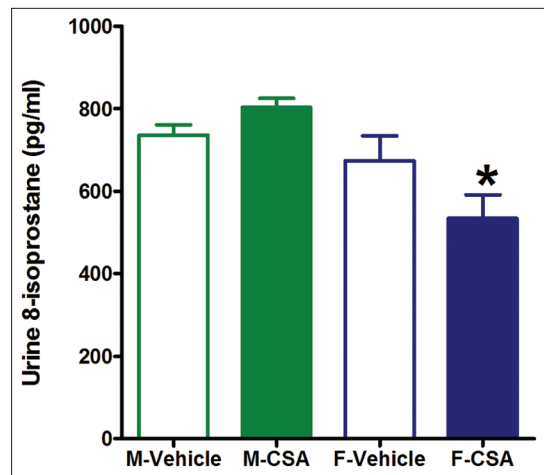


Figure 3: Urinary 8-isoprostane level in the studied rats groups (pg/ml)

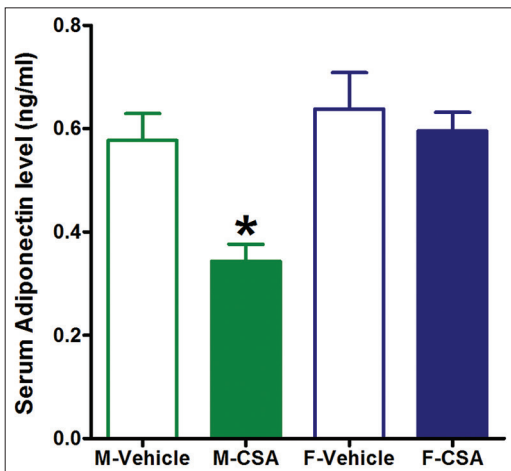


Figure 4: Serum adiponectin level in the studied rats groups ng/ml

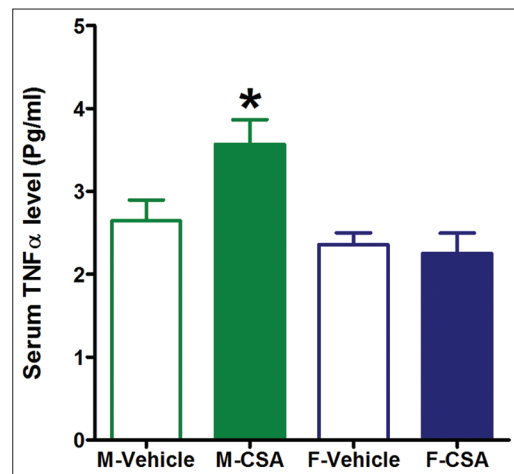


Figure 5: Serum tumor necrosis factor- α level in the studied rats groups (pg/ml)

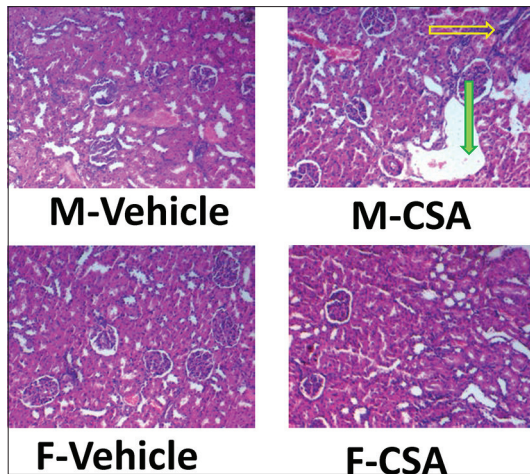


Figure 6: Histopathological findings of kidney tissue of the studied rats groups (H and E stained cross-sections)

renal impairment in CSA exposed rats. The role of oxidative stress in the pathogenesis of CSA-induced nephrotoxicity could be explained by many proposed evidence. CSA induces xanthine oxidase activity and increases the production of oxidants.^[22] Oxidative stress could induce mitochondrial damage as occurs in CSA-induced hepatotoxicity.^[23] Moreover, CSA provokes endoplasmic reticulum (ER) stress and consequently oxidative stress.^[24] This was confirmed previously as CSA upregulates expression of ER stress markers especially, immunoglobulin-binding protein, in renal tissue biopsies.^[25] Finally, in addition to the increased oxidative stress markers production, CSA administration is associated with a marked decrease in the total antioxidant capacity of the kidneys.^[26,27]

Mitochondrial and endoplasmic stress is implicated in the pathogenesis of CSA-induced renal impairment.^[17,28] CSA therapy is specifically reported to induce reactive oxygen species production in renal mesangial cells^[29] as well as it is also implicated in reducing renal antioxidant defense mechanism. These findings clarify the role of oxidative stress in medicating CSA-induced nephrotoxicity^[27] which comes in agreement with the result of the current study.

One of the important findings of the current study in the lowered adiponectin level in 40% of CSA exposed male progeny than the control male rats. This coincides with a study published in 2015 by Sahin *et al.*^[30] They reported that CSA induces higher adiponectin levels, endothelial dysfunction,^[31,32] and platelet activation without inducing platelet aggregation^[33] in their study. Adiponectin levels were elevated as an endothelial damage marker.^[32] This hypoadiponectinemia could be considered as a compensatory response to endothelial damage induced by CSA administration.^[30]

The anti-inflammatory role of adiponectin is explained by inhibiting endothelial expression of adhesion molecules, suppressing adhesion of the monocytes to the vascular endothelium, so it counteracts the inflammatory response of the endothelium.^[30]

While our finding was antagonized previously by Hjelmeseaeth *et al.*,^[34] They found a negative correlation between CSA administration and adiponectin level, but it did not reach the statistically significant level. They recommended a further investigation to confirm their results, but our study disproves it.

Moreover, TNF- α is found elevated in 30% of CSA exposed male progeny in our study. This in agreement with Schenk *et al.*,^[35] They reported that CSA induces the gene expression of renal tissue TNF- α and TNF- α -receptor family ligands as well as soluble form TNF- α receptors at mRNA and protein levels.

CSA-induced TNF- α higher expression could contribute in the induction of ER stress-related apoptosis,^[36] and it is enhanced sustained ER stress could induce apoptotic cell death in CsA-mediated renal damage.^[37] In addition, it is reported that TNF- α mediates caspase 3/7 activation.^[38]

The combined increased TNF- α level and lowered adiponectin level with the histopathological picture of increased aggregation of chronic inflammatory cells in renal tissue could prove the CSA-induced chronic renal inflammatory damage in our study. The inflammatory inducing effect of CSA is previously documented by Segarra Medrano *et al.*,^[39] Torres *et al.*,^[40] Saito *et al.*,^[41] Rodrigues-Diez *et al.*,^[42] and Koh *et al.*^[43]

Regarding the sex differences in the investigated biochemical markers in the current study, we confirm in this work, the previously published data in 2018 about the gender-specific CSA-induced renal damage.^[2] That is explained by the role that the gonadal hormones play in CSA-induced nephrotoxicity differences in male than female progeny. The explanation of such observation was based on the anti-inflammatory role of both estrogen and testosterone.^[44] CSA administration induces reduction of testosterone production in male^[45] while it induces an increase in estrogen production in female.^[46]

CONCLUSION

From the current study, it could be concluded that CSA could induce renal inflammation as well as oxidative stress that may explain the impaired renal function. The oxidative stress in proved by an increased level of urinary 8-isoprostane. The CSA-induced renal inflammatory damage is presented by increased TNF- α and lowered adiponectin level as well as the prominent histopathological inflammatory picture in the form of aggregation of the inflammatory cell with renal vascular congestion. The sex difference was a prominent finding in their vulnerability to CSA effects with male progeny are more affected than female.

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Conflicts of interest

There are no conflicts of interest.

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