Tropical Journal of Pharmaceutical Research July 2024; 23 (7): 1077-1082
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v23i7.5

Original Research Article

Roxadustat protects rats from cisplatin-induced acute kidney injury

Cüneyt Arıkan[1]*, Ejder Saylav Bora[2], Duygu Burcu Arda[3], Oytun Erbaş[4]
[1]Department of Emergency Medicine, Faculty of Medicine, Dokuz Eylül University, [2]Department of Emergency Medicine, Atatürk Training and Research Hospital, İzmir, [3]Department of Pediatrics, Gaziantep University, Gaziantep, [4]Department of Physiology, Demiroglu Bilim University, Istanbul, Turkey

For correspondence:* ***Email:* *cuneyt.arikan@deu.edu.tr;* ***Tel:*** *0090-5324507855*

Sent for review: 2 May 2023 *Revised accepted: 8 July 2024*

Abstract

Purpose: *To investigate the potential protective effect of roxadustat against cisplatin-induced acute kidney injury (AKI) by evaluating biochemical markers, inflammatory parameters, renal function tests, and histopathological changes.*
Methods: *Thirty female Wistar rats were randomized into control group, cisplatin with tap water group, and cisplatin with roxadustat group. Cisplatin-induced AKI was established by intraperitoneal injection of cisplatin at 10 mg/kg for seven days. Roxadustat was administered orally at 20 mg/kg/day to the treatment group. Blood and kidney samples were collected for biochemical and histopathological analyses respectively.*
Results: *Roxadustat treatment significantly reduced markers of renal injury (malondialdehyde (MDA), kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), transforming growth factor-beta 1 (TGF-beta1)), inflammatory cytokines (tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-18 (IL-18)) compared to the cisplatin group ($p < 0.005$). In addition, roxadustat treatment also improved renal function (blood urea nitrogen (BUN), serum creatinine (SCr)) compared to the cisplatin group ($p < 0.005$). Histopathological examination revealed a significant decrease in tubular epithelial necrosis and luminal necrotic debris in the roxadustat-treated group ($p < 0.005$). However, there was no significant difference in tubular dilatation and interstitial inflammation between groups ($p > 0.05$).*
Conclusion: *Roxadustat significantly prevents cisplatin-induced AKI by attenuating renal injury, reducing inflammation, and improving renal function. This evidence suggests that roxadustat may be a promising preventive option for patients receiving cisplatin chemotherapy.*

Keywords: *Roxadustat, FG-4592, Cisplatin, Acute kidney injury*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Several drugs or metabolites have the potential to directly affect the tubules of the nephrons. The use of nephrotoxic medication may damage structural elements of tubular cells, ultimately leading to poor renal function. The risk of tubular injury is increased when other medications that cause kidney damage are consumed simultaneously. Damage to the tubules results in atypical blood electrolyte levels. Acute tubular injury typically depends on dosage and duration

of exposure. When the kidney is exposed to higher doses of nephrotoxic drugs and for a longer duration, the likelihood of kidney damage increases [1].

Cisplatin has been used as a chemotherapeutic agent for many years in various types of cancer [2]. Since the kidneys predominantly excrete cisplatin, it may accumulate in the proximal tubules and cause nephrotoxicity. Cisplatin also disrupts electrolyte balance by stimulating the excretion of electrolytes such as magnesium. Its use is limited due to these side effects. Cisplatin induces kidney damage through complex mechanisms including apoptosis, inflammation, oxidative stress, and fibrogenesis. High concentrations of cisplatin cause necrosis in proximal tubule cells, while lower concentrations lead to apoptosis. Additionally, cisplatin inhibits antioxidant systems, and reduces the activity of superoxide dismutase, glutathione peroxidase, and catalase, thereby impairing renal function [3].

Another indirect mechanism for nephrotoxicity is the development of anemia. Erythropoietin is produced by peritubular interstitial cells. While anemia is a consequence of cisplatin-induced bone marrow suppression in both human and animal studies, it has been reported that the resulting kidney damage leads to erythropoietin deficiency, thus exacerbating the anemia [4]. Nephrotoxicity occurs in about one-third of patients receiving cisplatin, typically manifesting around ten days after treatment. Hydration, magnesium, and mannitol applications have been used to prevent cisplatin-induced nephrotoxicity [5]. However, there is insufficient recommendation and evidence to effectively prevent cisplatin-induced acute kidney injury (AKI).

Roxadustat exhibits favorable tolerability and effectiveness in preserving desired hemoglobin levels in individuals with chronic kidney disease (CKD) undergoing peritoneal dialysis, regardless of previous treatment. It is a well-tolerated alternative to epoetin alfa and other erythropoiesis-stimulating agents [6]. On the other hand, roxadustat is known as an anti-inflammatory and antioxidant agent via hypoxia-inducible factor (HIF). Hypoxia-inducible factor (HIF) regulates the expression of genes in response to decreasing oxygen levels, including those necessary for erythropoiesis and iron metabolism [7].

This study chose neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), and transforming growth factor beta (TGF-β) over other biomarkers for the following reasons. Neutrophil gelatinase-associated lipocalin (NGAL) is a glycoprotein that plays an important role in inflammation as an acute-phase protein and chemokine. It also acts as a regulator of iron homeostasis. Recent studies have confirmed that interleukin-18 (IL-18) plays a significant role in kidney damage caused by acute ischemia-reperfusion. Furthermore, it also has the potential to act as a mediator of tubular injury.

Published findings suggest that IL-18 is an early biomarker for diagnosing AKI [8]. Kidney injury molecule-1 (KIM-1) plays a role in cell phagocytosis, repair mechanisms, and antiinflammatory responses and is excreted in the urine. Nevertheless, KIM-1 stimulates the development of renal fibrosis, tubular apoptosis, and inflammatory response in individuals with CKD [9]. Transforming growth factor beta (TGF-β) has traditionally been recognized as a crucial factor in developing renal fibrosis. Furthermore, TGF-β also functions as a potent anti-inflammatory cytokine that suppresses renal inflammation [10].

Therefore, this study was aimed at investigating the potential protective effect of roxadustat against cisplatin-induced acute kidney injury (AKI).

EXPERIMENTAL

Animals

This study was conducted with 30 female Wistar rats (200–210 g). The rats were kept at a constant temperature of 22 °C and a 12 h light-dark cycle These animals were allowed to acclimatize for two weeks. During this period, all the rats were fed with normal feeds and water. Ethical approval was obtained from the Istanbul Bilim University Animal Ethics Committee (approval no. 1623125213). The study complied with the internationally accepted guide for the care and use of laboratory animals, published by the US National Institutes of Health [11] and conducted at the Bilim University Experimental Animal Center (Gebze, Istanbul).

Animal grouping

One rat from each of the three groups died. Thirty rats were included in the study, eight of which were in the control group (no drug administration). The remaining two groups received cisplatin (10 mg/kg) intraperitoneally (i.p.) throughout the study. Group 1 (10 rats) received 1 ml/kg/day of tap water orally, while Group 2 (10 rats) received 20 mg/kg/day of

roxadustat by oral gavage for seven days. The study was terminated by cervical dislocation with high-dose anesthesia. Blood was collected by cardiac puncture for biochemical examinations. Kidneys were removed for histopathologic and biochemical investigations.

Determination of plasma lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) were used to determine lipid peroxidation by measuring plasma malondialdehyde (MDA) levels. The plasma samples were mixed with TBARS and incubated at 100 °C for 1 h. After cooling, the samples were centrifuged at 3000 rpm, and the absorbance of supernatant was measured at 535 nm. Tetraethoxypropane was used as a calibration standard, and MDA was measured in nanomolar units.

Assessment of plasma tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), NGAL, KIM-1, and IL-18 levels

Plasma TNF-α, IL-6, NGAL, KIM-1, and IL-18 levels were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits.

Blood biochemistry studies

Blood samples were collected with a one-milliliter syringe, placed in Heparin-containing tubes, centrifuged at 3000 rpm for 10 min, and stored at -20 ^{o}C. Serum creatinine (SCr) and plasma blood urea nitrogen (BUN) levels were measured using an automated analyzer (Beckman-Coulter AU 640) with commercially purchased kits (Beckman-Coulter Inc., CA, USA).

Kidney biochemical analysis

After euthanasia, the protein content in kidneys placed at -20 °C was calculated using the Bradford technique [12]. Kidney levels of TGF-beta1 in tissue supernatants were measured using commercially available rat ELISA kits.

Histopathological examination of the kidney

The animals were sacrificed by cervical dislocation with high-dose anesthesia, and the kidneys were promptly extracted and preserved at -20 °C. The kidney was bisected along its axis. The lower half of the specimens underwent histopathologic and biochemical analyses. The lower portion of the kidney sections had a thickness of 4 µm and were stained with eosin and hematoxylin. An Olympus C-5050 digital camera was connected to an Olympus BX51 microscope to capture the sections.

The structural evaluation was performed using Image-Pro Express 1.4.5 (Media Cybernetics, Inc., USA). A naive observer examined ten microscopic fields per section under a magnification of 20. The kidney sections of each group of rats were analyzed using a semi-quantitative method. Quantification was based on the level of tubular epithelial necrosis, luminal necrotic debris, tubular dilatation, and interstitial inflammation. The evaluation process assigned scores based on the following criteria. A score of 0 was given for a range of 0 - 5, a score of 1 for a range of 6 - 20, a score of 2 for a range of 21 - 40, a score of 3 for a range of 41 - 60, a score of 4 for a range of 61 - 80, and a score of 5 for a range of 81 - 100.

Statistical analysis

Statistical Packages for Social Sciences (SPSS 15.0 IBM Corp, Armonk, NY, USA) was used for data analysis. Parametric variables were compared using analysis of variance and student t-test. Non-parametric variables were compared using the Mann-Whitney U test. In addition, the Shapiro-Wilk test was used to separate parametric and non-parametric data. $P < 0.05$ was considered statistically significant.

RESULTS

Biochemical parameters

Levels of MDA, KIM-1, NGAL, and TGF beta-1 in serum were significantly higher in the cisplatin and tap water group ($p < 0.001$). These markers significantly decreased in the roxadustat-treated group ($p < 0.05$). Additionally, inflammatory markers (TNF-α, IL-6, and IL-18) were significantly higher in the cisplatin + tap water group ($p < 0.05$), and their levels were significantly reduced after roxadustat treatment ($p < 0.05$). Renal function indices (SCr and BUN) were significantly higher in the cisplatin and tap water group ($p < 0.05$) and significantly lower in the roxadustat-treated group ($p < 0.05$; Table I).

Histo-architecture of the kidneys

Histopathologic examinations were conducted to evaluate the extent of damage and inflammation in the renal tubules. Tubular epithelial necrosis, luminal necrotic debris, tubular dilatation, and interstitial inflammation were significantly increased in the cisplatin + tap water group ($p < 0.001$). Roxadustat treatment significantly reduced the extent of tubular epithelial necrosis

and luminal necrotic debris ($p < 0.05$). There was no significant difference in tubular dilatation and interstitial inflammation dimensions of treated groups (Figure 1, Table 2).

DISCUSSION

Cisplatin, a chemotherapeutic agent used for many cancer treatments, has the most critical side effect of nephrotoxicity. Its most critical and proven effect is on renal tubules. Depending on the dose, its adverse effects on cells may cause oxidative stress, apoptosis, inflammation, and fibrosis [3]. This present study showed that roxadustat was effective in reducing inflammation and reversing renal injury. Mounting evidence indicates that renal hypoxia is present in AKI caused by different factors and has significant implications for both ischemic and toxic forms of acute renal failure. The activation of HIF has been recognized as a crucial mechanism by which cells adapt to low oxygen levels. Several prior studies have documented that pre-conditional activation of HIF protects against renal ischemia-reperfusion and cisplatin-induced injury [13]. Roxadustat significantly mitigated inflammation, oxidative stress, and tissue damage from hypoxia, improving the body's capacity to acclimate to high-dose exposure [14]**.** Moreover, roxadustat also mitigated doxorubicin-induced damage to the heart by increasing the expression of HIF-1 and its associated genes, possibly by inhibiting programmed cell death and reducing oxidative stress [15].

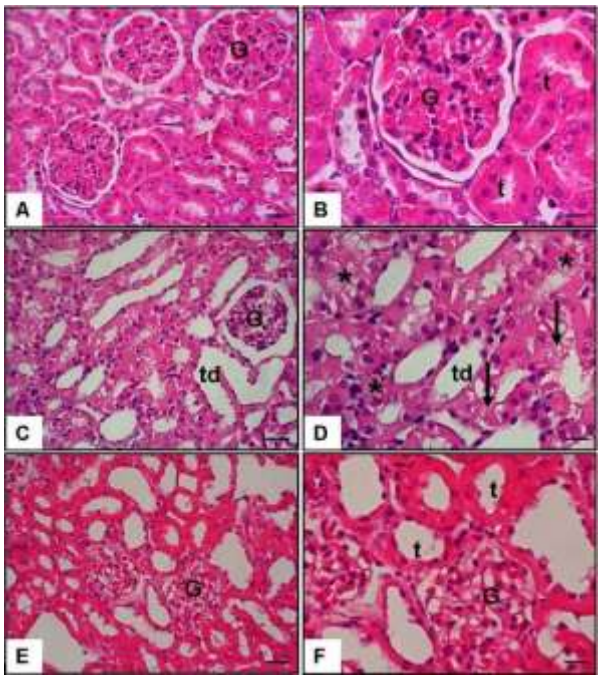

Figure 1: Kidney histopathology H & E (x 10 and x 40 Magnification), A-B: Control (glomeruli (G), tubules (t)), C-D: Cisplatin and tap water group showed kidney having an acute tubular injury findings with tubular cell necrosis (arrow), loss of brush borders & cellular detritus (*) and tubular dilatation (td), E-F: Cisplatin and roxadustat group have reduced acute tubular injury

Table 1: Biochemical parameters

Parameter	Control	Cisplatin+ tap water	Cisplatin+r oxadustat
Plasma MDA (nM)	48.6±2.1	103.2±2.8**	62.5±7.7$^{\#\#}$
Plasma TNF-alfa (pg/ml)	23.5±0.9	68.1±3.3*	42.7±1.8$^{\#}$
Plasma IL-6 (ng/ml)	1.38±0.3	93.1±4.2**	27.4±2.3$^{\#\#}$
KIM-1(pg/ml)	35.6±1.7	73.4±2.9**	55.2±4.7$^{\#}$
NGAL (pg/ml)	59.4 ± 2.3	122.6±3.5**	92.9±1.2$^{\#}$
IL-18 (pg/ml)	7.7±0.3	24.1±1.5*	13.3±0.9$^{\#}$
SCr (mg/dl)	0.42±0.05	2.12±0.1**	1.67±0.2$^{\#}$
BUN (mg/dl)	18.7±0.8	49.4±1.1*	33.8±0.5$^{\#}$
Kidney TGF-Beta1 Level (pg/mg protein)	14.3±1.6	81.6±2.5**	37.1±4.9$^{\#\#}$

Results were presented as mean ± SEM. *$P < 0.01$, **$p < 0.001$ compared to control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.001$ compared to cisplatin + tap water group

Table 2: Histopathological features

Parameter	Control group	Cisplatin+tap water group	Cisplatin+roxadustat group
Tubular epithelial necrosis	0.1±0.1	2.8±0.3*	1.2±0.2$^{\#}$
Luminal necrotic debris,	0.2±0.1	3.1±0.2*	1.7±0.3$^{\#}$
Tubular dilatation	0.1±0.1	2.9±0.3*	2.6±0.3
Interstitial inflammation	0.1±0.2	0.6±0.1*	0.5±0.1

Data were presented as mean ± SEM. *$P < 0.01$ compared to control group, $^{\#}p < 0.01$ compared to cisplatin + tap water group

Roxadustat alleviated alcohol-induced damage by suppressing hepatic lipid synthesis, attenuating inflammation, and mitigating oxidative stress. Results of this study revealed that kidney injury, which occurs due to the destructive effect of cisplatin, was decreased by roxadustat with antioxidant and anti-inflammatory effects. Another study revealed that the renal HIF system is critical in natural defense mechanisms against injury, and roxadustat reverses AKI [16]. Furthermore, it is uncertain whether roxadustat protects against kidney injury by inhibiting inflammation. The results of this study have provided a clear and promising method for treating AKI by counteracting the inflammatory response. Yang *et al* [7] revealed that roxadustat significantly improved cisplatin-induced AKI in mice by potently blocking NGAL and KIM-1, reduced inflammation and significantly improved renal function in mice which is in tandem with the findings of this present study [7]. Another study showed that roxadustat protects the kidneys from ischemia/reperfusion-induced AKI by suppressing kidney damage and inflammation [15]. In addition, roxadustat was reported to reduce kidney damage and prevent long-term fibrosis through antioxidant and anti-inflammatory effects in folic acid-induced kidney injury [16]. This suggests that roxadustat protected the kidneys through multiple mechanisms of action.

Histopathologic examination showed that tubular injuries decreased with roxadustat treatment, but the extent of interstitial inflammation did not change significantly. These results suggest that although roxadustat decreases cytokine levels in plasma, it does not affect the degree of inflammation in tissues. The first reason for this is the duration of treatment. In this study, examinations were performed after a seven-day treatment. In other studies, treatment periods ranged from 4 to 14 days [7,15,16]. The second reason may be the treatment onset. There is no evidence that roxadustat reduces interstitial inflammation histopathologically in cisplatin-induced AKI [7]. However, there is evidence that roxadustat reduces interstitial inflammation histopathologically in ischemia/reperfusion-induced AKI [16]. In this study, roxadustat treatment significantly improved renal function which has been reported in other studies [7,16,17]. Regardless of the cause of AKI (cisplatin, ischemia, or folic acid), renal function tests were significantly improved with roxadustat treatment. In another study, roxadustat reduced experimental pulmonary fibrosis severity by inhibiting TGF-1/Smad activation [18]. On the other hand, Wu et al. [19] showed that roxadustat reduces factors associated with renal fibrosis. In this present study, roxadustat demonstrated an anti-fibrotic effect by reducing TGF-1beta levels in the kidney. Furthermore, evaluation of the tubulo-epitelial damage recovery after administration revealed that roxadustat directly affects apoptosis [15]. Another indirect mechanism of cisplatin-induced nephrotoxicity is the development of anemia. Anemia occurs due to bone marrow suppression of cisplatin and the resulting kidney damage leading to erythropoietin (EPO) deficiency [4]. Roxadustat ameliorated anemia due to chronic renal failure [7,16]. It also prevented anemia by inducing EPO production via HIF and consequently protects patients from the nephrotoxic effect of cisplatin.

CONCLUSION

Roxadustat significantly inhibits cisplatin-induced AKI by reversing renal injury, reducing inflammation, and improving renal function in rats. Therefore, roxadustat treatment is a potential therapeutic option for patients undergoing cisplatin chemotherapy.

DECLARATIONS

Acknowledgements

None.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Oytun Erbaş, Ejder Saylav Bora, Duygu Burcu Arda and Cüneyt Arıkan, contributed equally during the study and made critical revisions related to the relevant intellectual content of the manuscript.

REFERENCES

1. *Wu H, Huang J. Drug-induced nephrotoxicity: Pathogenic mechanisms, biomarkers and prevention strategies. Curr Drug Metab 2018; 19(7): 559-567.*
2. *Gómez-Ruiz S, Maksimović-Ivanić D, Mijatović S, Kaluđerović GN. On the discovery, biological effects, and use of Cisplatin and metallocenes in anticancer chemotherapy. Bioinorg Chem Appl 2012; 2012: 140284.*
3. *McSweeney KR, Gadanec LK, Qaradakhi T, Ali BA, Zulli A, Apostolopoulos V. Mechanisms of cisplatin-induced acute kidney injury: Pathological mechanisms, pharmacological interventions, and genetic mitigations. Cancers (Basel) 2021; 13(7): 1572.*
4. *Wood PA, Hrushesky WJ. Cisplatin-associated anemia: an erythropoietin deficiency syndrome. J Clin Invest 1995; 95(4): 1650-1659.*
5. *Duffy EA, Fitzgerald W, Boyle K, Rohatgi R. Nephrotoxicity: Evidence in patients receiving cisplatin therapy. Clin J Oncol Nurs 2018; 22(2): 175-183.*
6. *Charytan C, Manllo-Karim R, Martin ER, Steer D, Bernardo M, Dua SL, Moustafa MA, Saha G, Bradley C, Eyassu M, et al. A randomized trial of roxadustat in anemia of kidney failure: SIERRAS study. Kidney Int Rep 2021; 6(7): 1829-1839.*
7. *Yang Y, Yu X, Zhang Y, Ding G, Zhu C, Huang S, Jia Z, Zhang A. Hypoxia-inducible factor prolyl hydroxylase inhibitor roxadustat (FG-4592) protects against cisplatin-induced acute kidney injury. Clin Sci (Lond) 2018; 132(7): 825-838.*
8. *Nisula S, Yang R, Poukkanen M, Vaara ST, Kaukonen KM, Tallgren M, Haapio M, Tenhunen J, Korhonen AM, Pettilä V, et al. Predictive value of urine interleukin-18 in the evolution and outcome of acute kidney injury in critically ill adult patients. Br J Anaesth 2015; 114(3): 460-468.*
9. *Song J, Yu J, Prayogo GW, Cao W, Wu Y, Jia Z, Zhang A. Understanding kidney injury molecule 1: a novel immune factor in kidney pathophysiology. Am J Transl Res 2019; 11(3): 1219-1229.*
10. *Gu YY, Liu XS, Huang XR, Yu XQ, Lan HY. Diverse role of TGF-β in kidney disease. Front Cell Dev Biol 2020; 8: 123.*
11. *National Research Council. Guide for the care and use of laboratory animals, National Academies Press. Washington, DC; 2010.*
12. *Kielkopf CL, Bauer W, Urbatsch IL. Bradford assay for determining protein concentration. Cold Spring Harb Protoc 2020; 2020(4): 102269.*
13. *Fähling M, Mathia S, Paliege A, Koesters R, Mrowka R, Peters H, Persson PB, Neumayer HH, Bachmann S, Rosenberger C. Tubular von Hippel-Lindau knockout protects against rhabdomyolysis-induced AKI. J Am Soc Nephrol 2013; 24(11): 1806-1819.*
14. *Guo Q, Li X, Li W, Wang R, Zhao A, Wang Z. A pharmacodynamic evaluation of the protective effects of roxadustat against hypoxic injury at high altitude. Drug Des Devel Ther 2023; 17: 75-85.*
15. *Long G, Chen H, Wu M, Li Y, Gao L, Huang S, Zhang Y, Jia Z, Xia W. Antianemia drug roxadustat (FG-4592) protects against doxorubicin-induced cardiotoxicity by targeting antiapoptotic and antioxidative pathways. Front Pharmacol 2020; 11: 1191.*
16. *Miao AF, Liang JX, Yao L, Han JL, Zhou LJ. Hypoxia-inducible factor prolyl hydroxylase inhibitor roxadustat (FG-4592) protects against renal ischemia/reperfusion injury by inhibiting inflammation. Ren Fail 2021; 43(1): 803-810.*
17. *Li X, Zou Y, Xing J, Fu YY, Wang KY, Wan PZ, Zhai XY. Pretreatment with roxadustat (FG-4592) attenuates folic acid-induced kidney injury through anti-ferroptosis via the Akt/GSK-3β/Nrf2 pathway. Oxid Med Cell Longev 2020; 2020: 6286984.*
18. *Huang H, Wang X, Zhang X, Wang H, Jiang W. Roxadustat attenuates experimental pulmonary fibrosis in vitro and in vivo. Toxicol Lett 2020; 331: 112-121.*
19. *Wu M, Chen W, Miao M, Jin Q, Zhang S, Bai M, Fan J, Zhang Y, Zhang A, Jia Z, et al. Anti-anemia drug FG4592 retards the AKI-to-CKD transition by improving vascular regeneration and antioxidative capability. Clin Sci (Lond) 2021; 135(14): 1707-1726. doi: 10.1042/CS20210100.*