Comparative Effects of Fedratinib and Upadacitinib on Renal Ischemia-Reperfusion Injury in Rat Models

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KEYWORDS
RIRI, Inflammation, NF-Kb, Apoptosis, JAK/STAT Signalling, Fedratinib,Upadacitinib, Cell Death Pathways, Rat Model

ABSTRACT
Renal ischemia-reperfusion injury (RIRI) is a severe condition that frequently occurs following kidney transplantation or surgical procedures affecting renal blood flow. Inflammatory responses, oxidative stress, and apoptotic pathways significantly contribute to RIRI. This study aimed to compare the renoprotective effects of Fedratinib, a JAK2 inhibitor, and Upadacitinib, a JAK1 inhibitor, in rat models of RIRI. Both inhibitors were administered one hour prior to ischemia induction, and the effects on renal function, inflammation, and cell death pathways were assessed. The findings revealed that both Fedratinib and Upadacitinib significantly reduced serum creatinine and urea levels, inflammatory cytokines (TNF-α, IL-6), and markers of apoptosis (BCL2/BAX) by suppressing the JAK/STAT signaling pathways. Histopathological analysis showed substantial reduction in renal tissue damage in the treated groups compared to controls. The study concludes that JAK inhibition by either drug provides significant renoprotection in RIRI, though with some differences in the degree of pathway inhibition and clinical implications.

1. Introduction

Ischemia-reperfusion injury (IRI) occurs when blood flow is restored to tissue following a period of reduced supply, leading to inflammation and damage to the affected tissue (1). This phenomenon can affect various organs, including the kidneys, and is often seen in clinical scenarios like organ transplantation and surgical procedures. Renal ischemia-reperfusion injury (RIRI) is characterized by the temporary loss of blood flow to the kidneys, followed by its restoration, which can cause inflammation, oxidative stress, and cell death. The severity of RIRI ranges from minor injuries that resolve spontaneously to severe damage requiring medical intervention.(2,3,4)

The kidneys are highly vascular organs with complex anatomy and physiology, making them particularly susceptible to IRI. Renal damage during IRI primarily affects four key structures: tubules, glomeruli, interstitium, and intra-renal blood vessels (5). The pathophysiology of RIRI involves multiple mechanisms, including ATP depletion, oxidative stress, activation of pro-inflammatory cytokines, and apoptosis, which collectively contribute to renal dysfunction. Pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 play a pivotal role in the inflammatory response during RIRI (4), while the JAK/STAT signaling pathway is crucial for the activation and progression of these cytokine-mediated processes.(6,7)

Fedratinib, a JAK2 inhibitor, and Upadacitinib, a JAK1 inhibitor, are therapeutic agents that have shown potential in mitigating RIRI by inhibiting the JAK/STAT pathway. This pathway's suppression reduces oxidative stress, inflammation, and apoptotic cell death, thus protecting renal function. Fedratinib, approved for the treatment of myelofibrosis, and Upadacitinib, used in autoimmune conditions like rheumatoid arthritis, offer targeted inhibition of JAK2 and JAK1, respectively.(8,9,10)

This study aims to compare the nephroprotective effects of Fedratinib and Upadacitinib in male rat models of renal ischemia-reperfusion injury, exploring their impact on renal function, inflammation, and apoptosis through the inhibition of the JAK/STAT signaling pathway. Understanding these

effects may lead to improved therapeutic strategies for managing RIRI and enhancing patient outcomes in clinical settings.

2. Materials and Methods

Study Location and Duration

The study was conducted at the research center of the College of Medicine, Kufa University, between May 2023 and January 2024.

Animal Preparation

Fifty healthy male Sprague-Dawley rats (100–150g) were obtained from the animal house of Kufa University, College of Science. The rats were housed under controlled conditions (25 ± 2°C, 12-hour light/dark cycle) with free access to standard rodent chow and water. The study was approved by the Animal Ethical Committee of Kufa University.

Study Design and Groups

Following a one-week acclimatization period, the rats were randomly divided into five groups (n = 10 each):

1. **Sham group:** Underwent anesthesia and surgical procedures without ischemia induction.
2. **Control group:** Induced ischemia-reperfusion injury (IRI) without treatment.
3. **Vehicle group:** Received vehicle (diluted DMSO) and IRI.
4. **Fedratinib group:** Received Fedratinib (30 mg/kg i.p.) one hour before ischemia induction and IRI.
5. **Upadacitinib group:** Received Upadacitinib (25 mg/kg i.p.) one hour before ischemia induction and IRI.

Experimental Procedures

The rats were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg). Ischemia was induced by clamping the renal pedicle for 30 minutes, followed by 90 minutes of reperfusion. The Sham group underwent the same surgical procedure without ischemia. Blood samples and kidney tissues were collected for analysis after reperfusion (11, 12.13).

Sample Preparation and Analysis

Blood samples were collected via cardiac puncture and centrifuged to isolate serum for urea and creatinine analysis. Kidney tissues were preserved in 10% formalin for histopathological analysis and in a tissue stabilizer for homogenization and PCR to assess levels of TNF-α, IL-6, NF-κB, Bax/Bcl2, and HMGB1.

Statistical Analysis

Data were analyzed using GraphPad Prism 9.3.1. Normality was assessed with the Shapiro-Wilk test, and group comparisons were made using One-Way ANOVA with Bonferroni post hoc tests. Differences were considered statistically significant at $p < 0.01$. Histopathological damage was scored based on the proportion of affected tubules.

3. Results and Discussion

Impact on Serum Urea Levels Serum urea levels significantly increased in the control and vehicle groups compared to the sham group. Both Fedratinib and Upadacitinib treatments significantly reduced serum urea levels compared to the control group, with no significant difference between the two treatments (Table 1).

Table 1 Mean level of serum urea (mg/dl) of the five animal groups at the end of the experiment; there were ten rats in each group

P1: control VS sham, P2: control VS vehicle, P3: control VS Fedratinib P4: control VS Upadacitinib, P5: comparison among two drugs.

Groups	Mean	SEM	P1	P2	P3	P4	P5
Sham	24.53	0.7476					
Control	76.70	2.409					
Vehicle	79.20	2.299	<0.01	>0.9999	<0.01	<0.01	>0.9999
Fedratinib	41.00	2.371					
Upadacitinib	41.40	2.083					

Impact on Serum Creatinine Levels Serum creatinine levels showed a significant rise in the control and vehicle groups compared to the sham group. Fedratinib and Upadacitinib significantly lowered serum creatinine levels compared to the control group, with no significant difference between the treatments (Table 2).

Table 2 Renal mean level of Serum Creatinine (mg/dl) of the five animal groups at the end of the experiment; there were ten rats in each group

P1: control VS sham, P2: control VS vehicle, P3: control VS Fedratinib P4: control VS Upadacitinib, P5: comparison among two drugs

Groups	Mean	SEM	P1	P2	P3	P4	P5
Sham	0.2576	0.01655					
Control	1.682	0.04402					
Vehicle	1.725	0.04696	<0.01	>0.9999	<0.01	<0.01	>0.9999
Fedratinib	0.4510	0.02263					
Upadacitinib	0.4210	0.03653					

Impact on Inflammatory Mediators

A. **TNF-α Levels** TNF-α levels were significantly higher in the control and vehicle groups compared to the sham group. Both Fedratinib and Upadacitinib reduced TNF-α levels significantly compared to the control, with no notable difference between the two treatments

(Table 3).

Table 3. Renal mean level of TNF-α (pg./ml) of the five animal groups at the end of the experiment; there were ten rats in each group

P1: control VS sham, P2: control VS vehicle, P3: control VS Fedratinib P4: control VS Upadacitinib, P5: comparison among two drugs.

Groups	Mean	SEM	P1	P2	P3	P4	P5
Sham	22.80	0.9852					
Control	86.94	3.840					
Vehicle	91.41	3.604	<0.01	>0.9999	<0.01	<0.01	>0.9999
Fedratinib	33.33	2.695					
Upadacitinib	33.09	2.187					

IL-6 Levels IL-6 levels increased significantly in the control and vehicle groups. Fedratinib and Upadacitinib treatments reduced IL-6 levels significantly compared to the control, with no significant difference between the treated groups (Table 4).

Table 4 Renal mean level of IL6 (pg./ml) of the five animal groups at the end of the experiment; there were ten rats in each group

P1: control VS sham, P2: control VS vehicle, P3: control VS Fedratinib P4: control VS Upadacitinib, P5: comparison among two drugs

Groups	Mean	SEM	P1	P2	P3	P4	P5
Sham	14.49	0.7980					
Control	76.49	2.845					
Vehicle	75.38	3.902					
Fedratinib	36.74	1.812	<0.01	>0.9999	<0.01	<0.01	<0.01
Upadacitinib	26.07	1.098					

HMGB-1 Levels HMGB-1 levels showed a significant increase in the control and vehicle groups. Both treatments significantly lowered HMGB-1 levels compared to controls, with no significant differences between Fedratinib and Upadacitinib (Table 3.10).

Table 3. 1 Renal mean level of HMGB-1 (pg./ml) of the five animal groups at the end of the experiment; there were ten rats in each group

P1: control VS sham, P2: control VS vehicle, P3: control VS Fedratinib P4: control VS Upadacitinib, P5: comparison among Two drugs

Groups	Mean	SEM	P1	P2	P3	P4	P5
Sham	11.69	2.041					
Control	80.75	5.361					
Vehicle	81.56	5.034	<0.01	>0.9999	<0.01	<0.01	>0.9999
Fedratinib	46.56	3.357					
Upadacitinib	47.00	3.011					

NF-κB Levels NF-κB levels were significantly elevated in the control and vehicle groups. Both treatments significantly reduced NF-κB levels compared to controls, with no difference between the two drugs (Table 5).

Table 5. Renal mean level of NF-kB (pg/ml) of the five animal groups at the end of the experiment; there were ten rats in each group

P1: control VS sham, P2: control VS vehicle, P3: control VS Fedratinib P4: control VS Upadacitinib, P5: comparison among two drugs

Groups	Mean	SEM	P1	P2	P3	P4	P5
Sham	22.07	1.553					
Control	182.0	7.224					
Vehicle	184.9	7.150	<0.01	>0.9999	<0.01	<0.01	>0.9999
Fedratinib	85.61	2.461					
Upadacitinib	64.62	7.238					

Impact on Apoptotic Parameters

A. **BAX Levels** BAX levels were significantly higher in the control and vehicle groups compared to the sham group. Fedratinib and Upadacitinib treatments significantly decreased BAX levels compared to controls, with no difference between the treated groups (Table 6).

Table 6. Renal mean level of BAX (pg/ml) of the five animal groups at the end of the experiment; there were ten rats in each group

P1: control VS sham, P2: control VS vehicle, P3: control VS Fedratinib P4: control VS Upadacitinib, P5: comparison among two drugs

Groups	Mean	SEM	P1	P2	P3	P4	P5
Sham	0.1517	0.01250					
Control	6.758	0.3224					
Vehicle	6.846	0.1446	<0.01	>0.9999	<0.01	<0.01	>0.9999
Fedratinib	1.773	0.1142					

Upadacitinib	1.773	0.1636

B. BCL-2 Levels BCL-2 levels increased significantly in the control and vehicle groups. Both treatments significantly reduced BCL-2 levels compared to controls, with no significant difference between Fedratinib and Upadacitinib groups (Table 7).

Table 7. Renal mean level of BCL2 (pg/ml) of the five animal groups at the end of the experiment; there were ten rats in each group

P1: control VS sham, P2: control VS vehicle, P3: control VS Fedratinib P4: control VS Upadacitinib, P5: comparison among two drugs.

Groups	Mean	SEM	P1	P2	P3	P4	P5
Sham	0.1681	0.01390					
Control	5.920	0.2687					
Vehicle	6.128	0.1078	<0.01	>0.9999	<0.01	<0.01	>0.9999
Fedratinib	1.582	0.1209					
Upadacitinib	1.556	0.1278					

PCR and Gene Expression

JAK1 Expression JAK1 expression significantly increased in the control and vehicle groups compared to the sham group. Both treatments reduced JAK1 expression, with Upadacitinib showing a significantly greater reduction than Fedratinib (8).

Table 8. Renal mean JAK1 expression (pg/mg) of the five animal groups at the end of the experiment; there were ten rats in each group

P1: control VS sham, P2: control VS vehicle, P3: control VS Fedratinib P4: control VS Upadacitinib, P5: comparison among two drugs

Groups	Mean	SEM	P1	P2	P3	P4	P5
Sham	13.88	0.2075					
Control	6.580	0.1373					
Vehicle	6.380	0.3720	<0.01	>0.9999	<0.01	<0.01	<0.01
Fedratinib	9.842	0.2497					
Upadacitinib	11.64	0.2838					

JAK2 Expression JAK2 expression was significantly elevated in the control and vehicle groups. Both Fedratinib and Upadacitinib lowered JAK2 expression compared to controls, with a significant difference observed between the two treatments (Table 9).

Table 9. Renal mean JAK2 expression (pg/mg) of the five animal groups at the end of the

experiment; there were ten rats in each group

P1: control VS sham, P2: control VS vehicle, P3: control VS Fedratinib P4: control VS Upadacitinib, P5: comparison among two drugs

Groups	Mean	SEM	P1	P2	P3	P4	P5
Sham	8.416	0.3634					
Control	4.442	0.1157					
Vehicle	4.200	0.08563	<0.01	>0.9999	>0.9999	<0.01	<0.01
Fedratinib	5.668	0.1663					
Upadacitinib	6.380	0.3720					

STAT3 Expression STAT3 expression significantly increased in the control and vehicle groups. Both treatments significantly reduced STAT3 levels compared to controls, with significant differences noted between Fedratinib and Upadacitinib (Table 10).

Table 10. Renal mean Stat3 expression (pg/mg) of the five animal groups at the end of the experiment; there were ten rats in each group

P1: control VS sham, P2: control VS vehicle, P3: control VS Fedratinib P4: control VS Upadacitinib, P5: comparison among two drugs

Groups	Mean	SD	P1	P2	P3	P4	P5
Sham	17.27	0.2295					
Control	9.054	0.2085					
Vehicle	9.136	0.1693	<0.01	>0.9999	<0.01	<0.01	<0.01
Fedratinib	12.61	0.2154					
Upadacitinib	13.83	0.2947					

Histopathological Findings

Sham Group The sham group exhibited normal renal anatomy and no significant damage

(Figure 1).

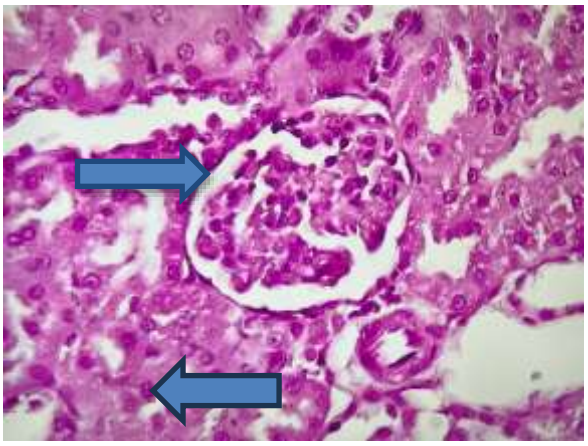

Figure 1 Sham group; A cross-section of the kidney (sham group) reveals a glomerulus (1) that is nearly normal and a renal tubule (2) that is completely normal. Hematoxylin and eosin stain (40X)

Control Group The control group showed significant renal damage, with 70% of rats having severe injury and 30% mild injury (Figure 2).

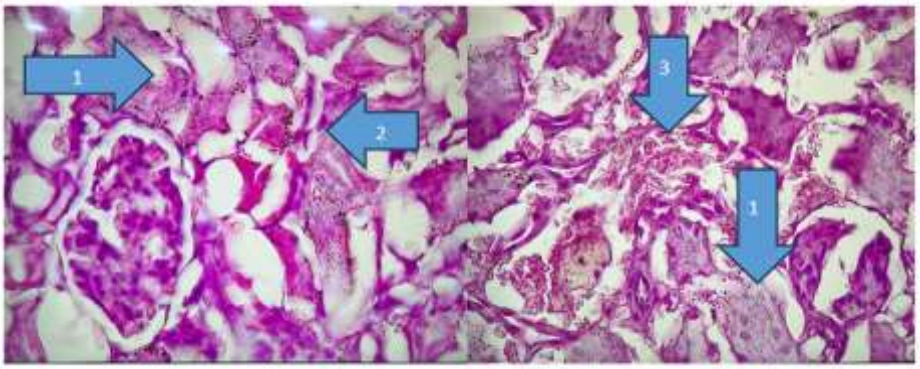

Figure 2: Ischemic change to the tubule Loss of tubular architect and loss of the nucleus in comparison to glomeruli (1), increased cytoplasmic eosinophilia and fragmentation together with neutrophilic inflammatory infiltrate (2) and marked hemorrhage and congestion

Control Vehicle Group Similar to the control group, 75% of the vehicle group had severe renal injury, and 25% had mild injury (Figure 3).

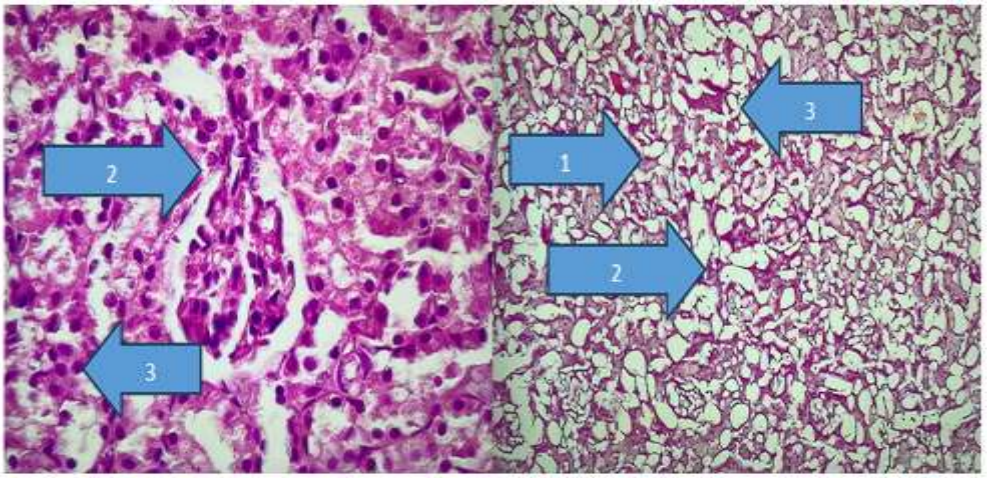

Figure 3: Section through kidney (DMSO group) loss of the nucleus (1) and renal glomerular tubular vasculation of the cytoplasm. (2) and marked haemorrhage and congestion (3). H and E stain (40X).

Fedratinib Treated Group Fedratinib treatment significantly improved renal damage, with 70% of rats showing moderate injury and 30% showing mild injury (Figure 4).

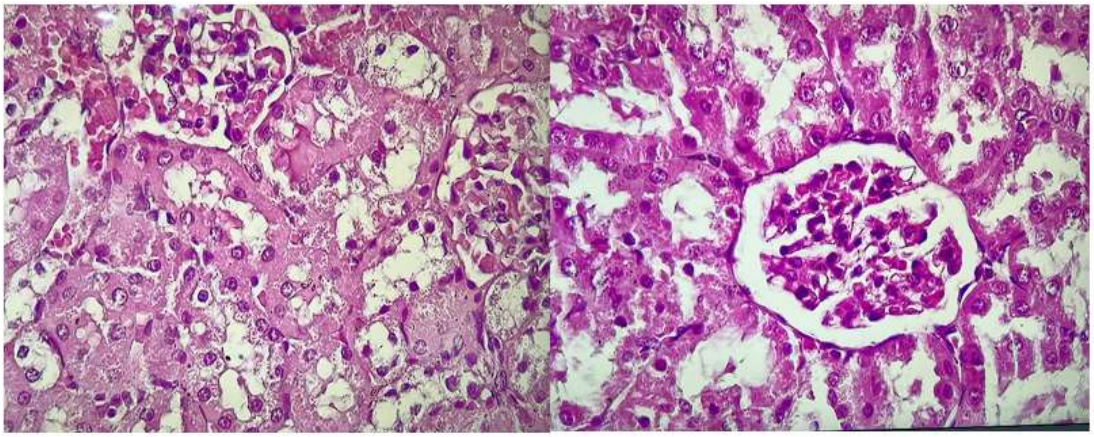

Figure 4: Mild congestion with a moderate ischemic change a lot of tubules kept their boundaries with a little Edematous change.

Upadacitinib Treated Group Upadacitinib treatment also improved renal injury, with 90% of rats showing moderate injury and 10% showing mild injury (Figure 5).

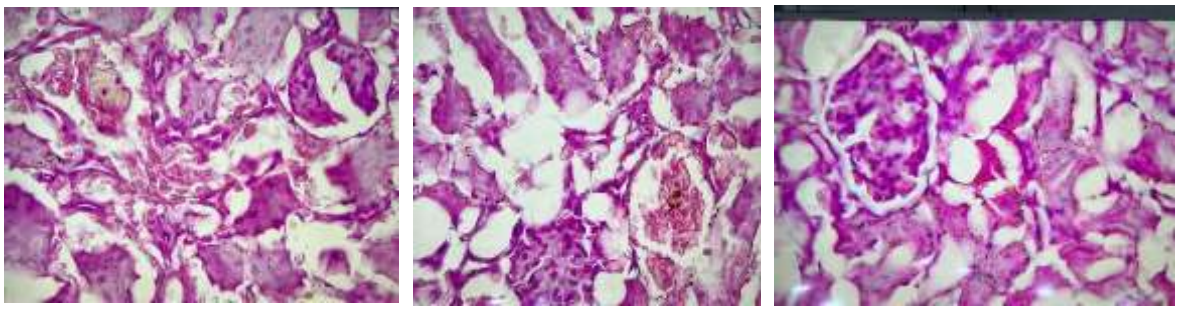

Figure 5: Moderate ischemia alterations and minor congestion with preserved glomeruli and tubules

Ischemic-reperfusion injury imposes acute stress on healthy tissues due to reduced oxygen and nutrient delivery, leading to a cascade of inflammatory responses. These responses can cause significant damage to both local and systemic tissues. Acute kidney injury (AKI) initiates a wide range of inflammatory reactions, primarily driven by local oxidative stress and lipid peroxidation. The objective of this study was to assess the therapeutic impact of upadacitinib, a JAK1 inhibitor known for its anti-inflammatory properties, by inhibiting the JAK-STAT pathway in a rat model of AKI (14).

The study results demonstrate significant increases in serum urea and creatinine levels in both the control and control vehicle groups, when compared to the sham group. This finding aligns with the recognized impact of ischemia-reperfusion injury (IRI) on kidney failure, which leads to a decrease in glomerular filtration rate (GFR) and the accumulation of nitrogenous waste compounds like urea and creatinine in the blood (15).

JAK inhibitors can provide a degree of protection against RIRI, resulting in a reduced severity of urea elevation compared to the control group (group 3). These effects are attributed to its anti-inflammatory and nephroprotective properties. Prior studies indicated that pre-treatment with upadacitinib significantly improved kidney function, as evidenced by reductions in blood urea nitrogen, serum creatinine, and albumin levels, as well as an increase in creatinine clearance. Upadacitinib also reduced renal inflammatory events, shown by decreased MDA and TNFα levels

(16). Upadacitinib effectively reduced histopathological structural damage in liver and kidney tissues. Confirmation of the renoprotective effect of upadacitinib was achieved through Western blotting analysis of NF-κB, supporting the above findings in our study (17).

A significant decrease in renal TNF-α levels was observed in the upadacitinib treatment group compared to the control groups (group 3). This suggests that the drug may have anti-inflammatory properties in the kidney, potentially by suppressing TNF-α production or activity (17,18). Upadacitinib is a Janus kinase (JAK) inhibitor that blocks signalling pathways involved in inflammation. TNF-α production is regulated by JAK-STAT signalling, so JAK inhibition could directly suppress its expression. Wang J and Macoritto M found that upadacitinib effectively reduced the expression of most TNF-IR increased modules in individuals who responded positively to JAK1 treatment. However, there were no changes observed in these modules among patients who were TNF-IR and received a placebo or among patients who were JAK1 inadequate responders (JAK1-IR) (19).

Bei Huang demonstrated that the overproduction of IL-6 activates the JAK/STAT pathway, leading to the formation of an inflammatory environment, which is responsible for initiating epithelial-mesenchymal transition (EMT). In "Acute Kidney Injury: Definition, Pathophysiology and Clinical Phenotypes," Konstantinos Makris explained that AKI induces harm to the kidneys and other organs through a combination of pro-inflammatory and oxidative stress-induced mechanisms. Cytokine levels (IL-1, IL-6, IL-10, and TNFα) in the blood and distant organs rise concurrently with the migration of white blood cells (neutrophils, lymphocytes, and macrophages) and increased oxidative stress (superoxide dismutase, malondialdehyde, and glutathione depletion). These findings are consistent with our observation of elevated levels of TNFα and IL-6 in the control group (group 3) (20). IL-6 plays a crucial role in linking the NF-κB signalling pathway with STAT3. NF-κB's target gene produces IL-6, and IL-6 and its receptor can efficiently activate STAT3 (6,21). Upadacitinib, along with tofacitinib, showed reversible inhibition of IL-6-induced pSTAT3 in a concentration-dependent manner (22,23). Additionally, upadacitinib and PD29 reduced inflammation by inhibiting IL-6 (16).

Andrassy M and Volz H discovered that HMGB1 plays a pivotal role in the early stages of ischemia/reperfusion (I/R) injury by binding to RAGE, which activates proinflammatory pathways and increases damage to heart muscle (24). MTX was shown to suppress the inflammatory signal in rheumatoid arthritis by disrupting the interaction between HMGB1 and the RAGE ligand at the molecular level, affecting the JAK/STAT pathway and suppressing the overproduction of TNF-α (25,26). Our study revealed that upadacitinib-induced JAK1 inhibition resulted in a decrease in HMGB1 levels, consistent with the above research findings.

Janus kinases (JAKs) are involved in the activation of NF-κB via signalling pathways such as TNF-α and IL-1. Inhibiting JAKs indirectly reduces the activity of NF-κB by blocking its activation through phosphorylation (27). The connection between the JAK/STAT signalling system and NF-κB is noteworthy (6,8). STAT3 regulates multiple factors essential to the tumour microenvironment. Following the identification of simultaneous activation of NF-κB and STAT3 in tumour cells (6), STAT3 is crucial in the activation of the NF-κB pathway.

A study by Anbar H and Shehab N. et al. examined the role of upadacitinib in reducing the harmful effects of cisplatin, measuring NF-κB expression in liver tissues and p-Akt levels, which serve as markers for cellular apoptosis response. The data suggest that cisplatin administration increased the expression of NF-κB p65 proteins compared to the control group (group 3). These effects were suppressed by upadacitinib and losartan, aligning with our finding that upadacitinib significantly reduced NF-κB (17).

Chai Y and Zhu K reported that dexamethasone might mitigate CP-induced AKI by reducing ER stress-induced apoptosis, partly via the α2AR/PI3K/AKT signalling pathway (15). Bax expression in human CD34+ hematopoietic cells is tightly controlled by its upstream activator, the JAK/STAT

signalling pathway. The study found that JAK inhibitors counteracted the effect of IL-6 or IL-3 on Bax transcription and Bcl-2 expression in human CD34+ hematopoietic cells, with curcumin showing greater potency than AG490 in suppressing IL-6-induced STAT3 phosphorylation (28). Our research supported these findings by showing a significant drop in Bax and Bcl-2 when the JAK/STAT pathway was inhibited.

Karjalainen R and Pemovska T et al. explained that substances produced by bone marrow stromal cells activate alternative signalling pathways, which cause resistance in bone marrow stroma conditions. This resistance signifies a change in cell survival dependence from BCL2 to BCLXL. Remarkably, the BCL2 inhibitor venetoclax's resistance was effectively countered by the JAK1/2 inhibitor ruxolitinib, indicating that JAK inhibitors can overcome resistance caused by BM stroma in AML (29). Moreover, AK activation promotes cell survival and might influence BCL2 expression or activity. Alternatively, by inhibiting JAK activity with JAK inhibitors, the BCL2-dependent survival of leukaemia cells may be indirectly reduced, explaining their vulnerability to BCL2 inhibitors. Takei H and Coelho-Silva J articulated this concept (30). Yuan X and Ni L demonstrated that the selective JAK1 inhibitor (JAK1i, upadacitinib) decreased Bcl-xL expression in SNK-6 cells in a dose- and time-dependent manner, both at protein and mRNA levels. Conversely, the JAK2 inhibitor (JAK2i, fedratinib) was significantly less effective in controlling Bcl-xL expression. JAK1 inhibitors, and to a lesser extent JAK3 inhibitors, had more pronounced effects compared to JAK2 inhibitors (31).

The JAK-STAT pathway facilitates signal transduction from extracellular ligands, such as various chemokines and cytokines. While these responses are typically seen in lymphoid cells, they also occur in kidney cells such as podocytes, mesangial cells, and tubular cells. Aberrant JAK-STAT pathway expression and signalling are observed in both human and animal models of various chronic renal diseases. The upregulation and increased function of JAK1, JAK2, and STAT3 contribute to the development of diabetic nephropathy, and their suppression appears to alleviate the disease (32). The JAK/STAT pathway has been implicated in both protecting and injuring cells (33). These findings corroborate our studies, which showed that reducing JAK1 expression can protect against RIRI. Additionally, JAK1 suppression enhances the resistance of vascular smooth muscle cells to H2O2-induced apoptosis. Recent studies have shown a correlation between JAK1 and tubular cell damage (33). Recent research suggests that JAK inhibitors, a class of antiviral drugs, may effectively mitigate acute kidney injury in COVID-19 patients by inhibiting Janus kinase enzymes in lymphocytes (34).

Park J and Yoo K found that STAT3 inhibition in vitro decreased fibrosis and cell death in human tubular epithelial cells subjected to 72 hours of hypoxia. It also reduced inflammation regulated by pSTAT3α. Furthermore, elevated phosphorylated STAT3 (pSTAT3) levels were observed in both human acute tubular necrosis and chronic kidney disease tissues. The progression of IRI is associated with STAT3 activation, and STAT3α may play a crucial role in this process

4. Conclusion

Upadacitinib, a JAK1 inhibitor, and Fedratinib, a JAK 2 inhibitor, led to a considerable decrease in RIRI as compared to the control group. The observed protective effect may be attributed to the drugs' ability to inhibit the JAK/STAT signalling pathway, leading to reduced levels of inflammatory markers (IL-6, TNF-α, HMGB1), suppressed activation of NF-κB, and decreased apoptosis.

Recommendation for future work

Further investigation is required to assess the efficacy and safety of upadacitinib and fedratinib in larger animal models, employing stronger functional assessments, and potentially progressing to clinical trials with testing of another pathways that may influence the JAK/STAT pathway, such as pi3/Akt.

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