



Etanercept Mitigated Renal Injury in Male Rats Undergoing Global Renal Ischemia-Reperfusion

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Abstract: **Introduction:** The kidneys are vulnerable to injury from ischemia-reperfusion (IR), a process that triggers inflammation and apoptosis, primarily mediated by tumor necrosis factor (TNF)-alpha. Numerous studies have investigated renal damage in this context. Etanercept, a soluble receptor for TNF-alpha, has demonstrated anti-inflammatory and anti-apoptotic properties. This study aims to assess the potential of etanercept in mitigating experimental renal IR injury and its capacity to protect against widespread renal ischemia/reperfusion injury. **Methods:** Male Sprague-Dawley (SD) rats were classified into four groups: sham, DMSO-treated, etanercept-treated, DMSO-treated IR, and etanercept-treated IR groups. After 24 hours following IR injury, renal levels of TNF-alpha and TLRs (Toll-like receptors) were assessed using ELISA and IHC methods, respectively. Histopathological analysis was employed to quantify the extent of renal cell injury. **Results:** Etanercept treatment significantly lowered tissue levels of TNF-alpha and TLRs in IR-damaged rats compared to DMSO-treated IR rats. Kidneys of DMSO-treated IR rats exhibited substantially elevated levels of TNF-alpha and TLRs when compared to DMSO-treated sham rats. Conversely, etanercept-treated IR rats displayed significantly reduced levels of TNF-alpha and TLRs compared to DMSO-treated IR rats. Pre-treatment with etanercept significantly alleviated the extent of damage in IR-injured rats compared to the control and DMSO groups. Etanercept further promoted the downregulation of TLRs and TNF-alpha, thereby enhancing resistance to renal damage during IR. **Conclusion:** In conclusion, etanercept shows promise in providing protection against renal ischemia-reperfusion injury by mitigating inflammation and apoptosis, as evidenced by reductions in TNF-alpha and TLR levels. This suggests its potential as a therapeutic intervention to mitigate renal damage resulting from ischemia-reperfusion injury.

Key Words: Ischemia-reperfusion; apoptosis; TNF-alpha; etanercept; inflammation

I. INTRODUCTION

Chronic renal disease ranks as the tenth leading cause of mortality, affecting around 10% of the population, and its prevalence is on the rise due to increased rates of diabetes and hypertension [1]. In cases of chronic kidney disease, end-stage renal disease marks its culmination, where kidney transplantation emerges as the optimal treatment option offering better life quality than dialysis. Kidney ischemic-reperfusion damage (IRI) [2], a primary pathophysiological process responsible for graft rejection and failure post-kidney transplantation, is a significant complication [3]. Moreover, IRI is a major contributor to acute kidney injury [4], causing approximately 1.7 million deaths annually and affecting approximately thirteen million individuals globally [5].

In urological procedures such as kidney transplantation, renal artery surgical revascularization, and partial nephrec-

tomy, renal ischemia-reperfusion (I/R) injury is a significant concern. The clinical impact of I/R injury stems from its association with high mortality and acute renal failure [6]. Toll-like receptors (TLRs), a class of cell membrane receptors constituting the innate immune system, play a crucial role in the initial response to tissue injury. TLR2 and TLR4 are constitutively expressed on renal epithelium, with their expression increasing following renal ischemia/reperfusion (I/R) injury [7]. Additionally, there was no significant difference observed between TLR-gene-targeted and control groups in the count of apoptotic tubular cells or the nuclear translocation of nuclear factor kappa-B (NF- κ B). Tumor necrosis factor-alpha (TNF- α), a potent proinflammatory cytokine, has diverse impacts on immunity, inflammation, cell differentiation, and apoptosis [8].

Mounting evidence implicates TNF- α and other inflam-

matory mediators in drug-induced liver damage, where inflammatory cells and cytokines contribute significantly to drug toxicity control. Studies have indicated that anti-TNF- α antibodies did not reduce drug-induced liver damage when administered 1 hour prior to a challenge, but their effect was pronounced when administered 2 or 8 hours later [9], [10]. Etanercept (IFX), a TNF- α receptor blocker, emerged as a therapeutic agent for TNF- α -mediated diseases. In liver damage patients, it binds and neutralizes TNF- α . Numerous studies have shown that IFX reduces cellular damage by lowering TNF- α levels and free radical production [11].

II. MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

After three weeks of acclimatization, male Sprague-Dawley (SD) rats were divided into four groups:

- 1) Sham group: Rats underwent general anesthesia without bilateral renal artery occlusion [2].
- 2) Control group (ischemic-reperfused): Bilateral renal ischemia was induced by clamping renal pedicles for 30 minutes, followed by 1 hour of reperfusion without drug treatment [2].
- 3) Vehicle group: Rats underwent the same surgical procedure as the control group but received the vehicle of drugs, NaCl, intraperitoneally (i.p) 30 minutes before ischemia [12].
- 4) Etanercept group: Rats underwent the surgical procedure of the control group along with the administration of Etanercept at 50 mg/kg intraperitoneally (i.p) 30 minutes before ischemia [13].

At the end of reperfusion, the animals were euthanized via decapitation under general anesthesia, and brains were isolated for analysis [14], [15].

PREPARATION OF DRUGS

Etanercept was dissolved in 0.9% normal saline and administered at a dose of 50 mg/kg i.p. 30 minutes before ischemia. Etanercept was prepared immediately before injection [12], [16].

INDUCTION OF GLOBAL BRAIN ISCHEMIA

General anesthesia was induced with intraperitoneal administration of ketamine (80-100 mg/kg) and xylazine (8-10 mg/kg). Bilateral renal ischemia was induced by clamping renal pedicles for 30 minutes, followed by 1 hour of reperfusion to induce global renal ischemia/reperfusion injury [18], [19].

After 1 hour of reperfusion, rats were euthanized, and kidney samples were collected for subsequent analysis [4], [20].

HISTOPATHOLOGY

After reperfusion, renal tissues were embedded in a paraffin block, fixed in 10% formalin, and sectioned into 5 μ m-thick slices. These sections were stained with hematoxylin-eosin

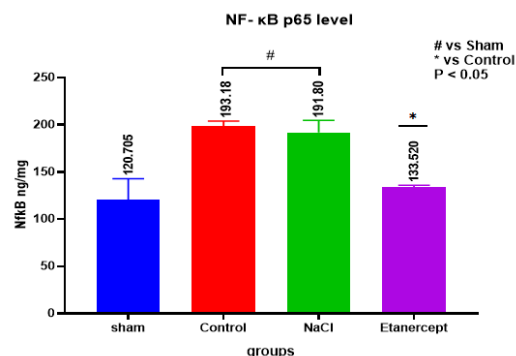


FIGURE 1: The difference in NF- κ B p65 levels among groups. Administration of Etanercept drugs 30 min. before ischemia was reduced NF- κ B p65 level (ng/mg) about 31% than the control group

(H&E), and subsequently examined under a microscope by a pathologist. The pathological scoring scale used in this study is as follows: [19] Normal (0): Absence of edema, RBCs, and eosinophilic neurons. Slight (1): Presence of edema or eosinophilic neurons. Moderate (2): Presence of edema, eosinophilic neuronal population, and a minor RBC population. Severe (3): Presence of necrosis, edema, eosinophilic neurons, and RBCs.

SEM STUDY

The morphological structure of the formulated cefdinir-loaded FMWCNT (F-CEF FMWCNT and FMWCNTs) was studied using scanning electron microscopy (SEM). Samples were prepared and analyzed using an SEM (JEOL, JSM-6360A, Tokyo, Japan) operating at a 10 kV acceleration voltage [19].

MEASUREMENT OF TLR2 AND TLR4 LEVELS, HO-1 VIA IMMUNOHISTOCHEMISTRY

Tissues collected from both untreated and treated groups were subjected to cell counting using TLR2 and TLR4 antibodies based on the manufacturer's protocol. The immunohistopathological scoring scale was calculated using the following equation [21]–[25].

III. RESULTS

MODULATION OF NF- κ B P65

The NF- κ B p65 level in renal tissues from all groups was assessed using the ELISA technique at the end of the experiment. I/R damage led to an elevation in NF- κ B p65 levels. Etanercept-treated groups exhibited significantly lower NF- κ B p65 levels ($p < 0.05$) compared to control groups (166.18 ± 7.73). There was no significant difference in NF- κ B p65 levels between the Etanercept-treated groups and the sham groups ($p > 0.05$). Refer to Table 1 and Figure 1. The difference in NF- κ B p65 levels between the vehicle group and the control group was negligible.

Groups n=4	Mean ± Std. Error	95% Confidence Interval for Mean		P value
		Lower	Upper	
sham	120.62 ± 22.17	98.45	142.79	
control	193.18 ± 5.73	192.45	203.91	# P<0.05
NaCl (vehicle)	191.80 ± 12.68	179.12	204.48	# P<0.05
Etanercept	133.52 ± 2.32	131.2	135.84	* P<0.05

TABLE 1: NF- κB p65 levels (ng/mg) of four experimental groups. The data expressed by one-way ANOVA. # vs sham, * vs control

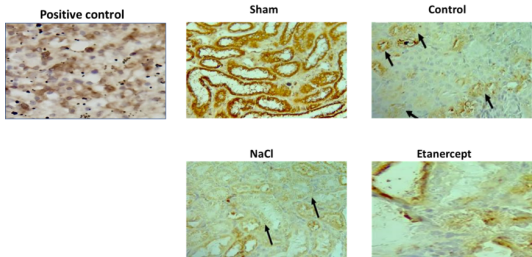


FIGURE 2: Normal human spleen (positive control) and the difference in TLRs levels among groups. Administration of Etanercept drugs 30 min. before ischemia was reduced level of TLRs. (X 400)

MODULATION OF TLR2 AND TLR4

Using the immunohistochemical approach, we assessed the levels of TLRs in renal tissues across all groups at the experiment’s conclusion. The IHC analysis revealed alterations in the levels of TLR2 and TLR4 following a 30-minute ligation followed by a 60-minute reperfusion. While etanercept did not induce TLR expression in renal tissues, it was observed in the control and vehicle groups. Notably, strong TLR expression was induced in the control positive tissue (normal spleen tissue). Refer to Figure 2.

PHARMACOLOGICAL ETANERCEPT REDUCED NECROSIS, HEMORRHAGE, DARK EOSINOPHILIC NEURONS, AND EDEMA CAUSED BY BRAO

Microscopic examination of renal tissue revealed tissue damage resulting from the closure of renal arteries for 30 minutes, followed by 60 minutes of reperfusion. The extent of damage was characterized by the presence of edema, dark eosinophilic neurons, hemorrhagic regions, or necrosis. An expert pathologist examined the renal tissue from each experimental group post-experimentation.

Histological analysis demonstrated significantly lower renal tissue damage scores ($p < 0.05$) in the Etanercept groups compared to the control group. While the histopathological scores of the control and vehicle groups were notably elevated ($p < 0.05$) in comparison to the sham group, the Etanercept groups exhibited a significant reduction in scores ($p < 0.05$) compared to the control group. Refer to Table 2 and Figure 3. No significant differences in scores ($p > 0.05$) were observed between the Etanercept groups and the sham group, or between the control group and the vehicle group.

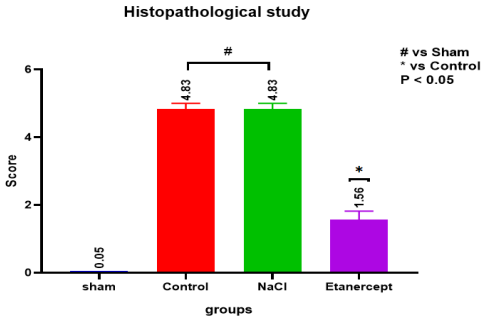


FIGURE 3: The histopathological score among groups. The control and vehicle groups were increased damage score of 100 % than the sham group. Administration of Etanercept drugs 30 min. before ischemia was reduced the score 65 percent than the control group

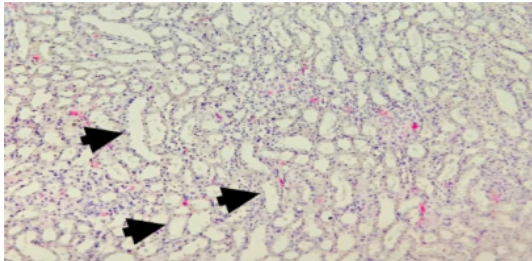


FIGURE 4: The typical architecture is visible in the photomicrograph of a part for the fake group. Hematoxylin and eosin was used to stain the section. (X 100)

SHAM GROUP

A cross-sectional view of the rat kidney from the control group exhibited a normal structure, devoid of any signs of bleeding or dark eosinophilic neurons.

CONTROL AND VEHICLE GROUPS

A cross-sectional view of the rat renal tissue from the control and vehicle groups revealed an abnormal structure characterized by features such as edema, necrosis, dark eosinophilic neurons, and hemorrhage.

ETANERCEPT TREATED GROUP

Treatment of rats with pharmacological anti-TNF-α led to an improvement in renal injury when compared with the control and vehicle groups.

Groups n=4	Mean $\pm$ Std. Error	95% Confidence Interval for Mean		P value
		Lower	Upper	
sham	0.05 $\pm$ 0.00	0.00	0.00	
control	4.83 $\pm$ 0.17	2.40	3.26	# P<0.05
NaCl (vehicle)	4.83 $\pm$ 0.17	2.40	3.26	# P<0.05
Etanercept	1.56 $\pm$ 0.26	0.34	1.66	* P<0.05

TABLE 2: Renal damage score of four experimental groups. The data expressed by one-way ANOVA. # vs sham, * vs control

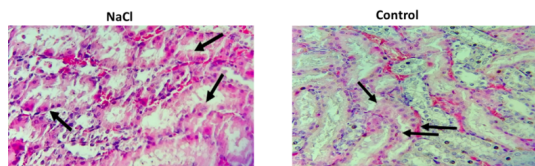


FIGURE 5: Photomicrograph of a renal section for the control and vehicle groups. The sections showed edema, hemorrhage, and necrosis. The section stained with Hematoxylin and Eosin (X 100)

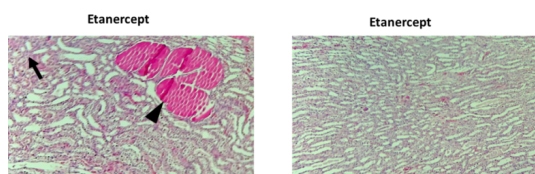


FIGURE 6: Photomicrograph of renal section in Etanercept group. The section stained with Hematoxylin and Eosin (X 100)

IV. DISCUSSION

By reducing renal tubular damage, apoptosis, and inhibiting TLR increases, we showed that etanercept guards against renal IR harm. Etanercept reduced the expression of TLRs, TNF- α , and damage score showed that this protection was predominantly brought about by the prevention of apoptosis and inflammation. The activation of apoptosis and neutrophil-mediated inflammatory injury may be the mechanisms through which IR-induced renal TNF- α expression damages renal cells [22], [23].

TNF- α is a proinflammatory cytokine that has the power to increase both its own and other crucial genes for the inflammatory response. Vascular inflammation results from the binding of TNF- α to its receptor, which also triggers the release of additional cytokines such as MCP-1, interleukin (IL)-6, IL-8, platelet-derived growth factor (PDGF), and cell adhesion molecules [14]. In the current investigation, we found that rats treated with etanercept had considerably lower TLR levels than rats treated with DMSO, demonstrating the anti-inflammatory potential of etanercept in renal IR injury. Apoptosis is one of the most significant mechanisms of cell death with renal IR injury in cultured renal tubular cells and isolated kidneys [24]. Comparing the levels in the DMSO-treated IR rats to those in the etanercept-treated IR rats showed that etanercept had an anti-apoptotic effect on renal IR injury. Furthermore, IR rats treated with etanercept suf-

fered considerably less renal cell damage than those treated with DMSO.

V. CONCLUSION

Etanercept exhibits a renoprotective effect against renal I/R injury by down-regulating TNF- α protein and inflammation.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this investigation.

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