

Anti-Oxidant Effect of Infliximab and DMF During Ischemia Reperfusion Induce Liver Injury in Male Rat

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Abstract

Background: Ischemia-reperfusion injury (IRI) to the liver causes inflammatory and oxidative stress events, as well as hepatocyte apoptosis and liver injury. In this study, we investigated whether infliximab, DMF, and their combination reduced ROS production in rat livers and played a protective role in the pathogenesis of I/R-induced liver injury in rats.

Aim: study the role of infliximab, DMF, and their combination in hepatic I/R injury. **Methods:** 5 groups of 40 male rats were randomized in the following groups: G1; sham; G2 control; ischemia-reperfusion (I/R): Rats were subjected 60-minute ischemia then 90-minute reperfusion., G3: I/R+IFX (I/R+IFX peritoneally administration of 7 mg/kg body weight 72 hrs. before I/R), G4; I/R+DMF (I/R+DMF peritoneally at a dose of 30 mg/kg body weight on 72 hrs. before I/R), and G5; I/R + Comb (IFX peritoneally 7 mg/kg +DMF 30 mg/kg body weight).

Results: Infliximab, DMF pre-treatment (7 g/kg, 30 mg/kg body weight, respectively), and their combination for 3 days before ischemia lowered ALT, AST, and TNF- α in the rat liver, while elevated GSH-Px level, and reduced liver injury. Pretreatment with the same combination dramatically lowered ALT, AST, and TNF- α and increased the level of GSH-Px.

Conclusion: Infliximab is a powerful TNF- α blocker and DMF is a master oxidative stress regulator. Both drugs limit ROS release and cell death signaling which protects the hepatocytes from injury during liver ischemia reperfusion.

Keywords: Infliximab, DMF, Hepatotoxicity, Ischemia-Reperfusion Injury, And TNF-A

INTRODUCTION

Patients undergoing difficult liver surgery or suffering from abdominal trauma frequently have hepatic ischemia-reperfusion damage (IRI), which has a high morbidity rate. The main causes of liver IRI are inflammatory and oxidative stress events [1]. A great number of studies have demonstrated that reactive oxygen species (ROS) are primarily responsible for microvascular and parenchymal damage, which is regarded as a key risk factor for IRI. ROS, being a byproduct of cellular metabolism, can be managed at normal levels under normal conditions. When IRI occurs, however, mitochondrial oxidative phosphorylation is hindered.

Furthermore, oxidative respiratory chain damage results in enormous ATP consumption and high levels of ROS [2]. Furthermore, excessive free radicals cause mitochondrial structure, function alteration, which leads to cell necrosis and apoptosis, all of which increase morbidity and mortality [3]. TNF- α regulates inflammation, cell death, and proliferation by activating multiple intracellular pathways.

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These pathways are causally connected to liver injury caused by concanavalin A, TNF- α , and ischemia-reperfusion, as well as liver regeneration, hepatic damage, and mortality. In view of recent discoveries, TNF- α and ROS inhibitors may be interesting new strategies for treating hepatic ischemia-reperfusion injury. TNF- α binding blocker monoclonal chimeric immunoglobulin G1 (IgG1) is infliximab [4]. It inhibits the production of several proinflammatory cytokines, including IL-1b, IFN-g, IL-2, TNF-a, IL-6, and IL-17 [5]. It may also diminish cell infiltration by blocking the TNF receptor, which causes immune cells to lyse and trigger death in activated macrophages and T cells [6]. DMF, Dimethyl Fumarate, was newly approved by the FDA [7]. DMF has antioxidant and anti-inflammatory drug that works by suppressing the NF- κ B pathway, which in turn suppresses downstream pro-inflammatory signaling pathways [8]. Furthermore, it modulates the GSH system and improves cellular response to elevation of ROS. The DMF's molecular method of action was Nrf2 activation and NF- κ B suppressing activities of, which plays a significant role in inflammation suppression. DMF disrupts the Keap1-Nrf2 complex, resulting in Nrf2 translocation to the nucleus [9].

PURPOSE OF STUDY

The purpose of the study was studying the role of infliximab, DMF, and their combination in hepatic I/R injury.

MATERIALS AND PROCEDURES

Animals

Male albino Wistar rats weighing 225-275 g were utilized. All of the animals were kept in optimal conditions (temperature, humidity, 12/12 dark-light cycle) and were fed a regular pellet diet and water ad libitum. Karbala University's Ethic Committee for Animal Research authorized all experimental protocols. 5 groups of 40 male Wistar albino rats were categorized, each consisting of eight rats: G1; sham: Rats were subjected to the identical treatment in a sham operation, but the liver lobe pedicle was not clamped. G2 control; ischemia-reperfusion (I/R): Rats were subjected 60-minute ischemia then 90-minute reperfusion, G3: I/R+IFX (I/R+IFX peritoneally administration of 7 mg/kg body weight 72 hrs. before I/R), G4; I/R+DMF (I/R+DMF peritoneally at a dose of 30 mg/kg body weight on 72 hrs. before I/R), and G5; I/R + Comb. (IFX peritoneally 7 mg/kg +DMF 30 mg/kg body weight)

[10]. After 90 minutes of reperfusion, blood collection. hepatic tissue samples were taken from the left lobe for histological study.

TNF- α , aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glutathione peroxidase (GSH-Px)

TNF- α was measured in serum samples using the ELISA method, as directed by the manufacturer. GSH-Px activity was determined using the Paglia et al method [11], and serum AST and ALT activities were determined using a spectrophotometric method using an autoanalyzer (Roche Diagnostic Ltd., West Sussex, UK).

Histopathological study

10% neutral formaldehyde was used to fixed liver tissue fixed for 24 hours, then before being embedded in liquid paraffin, it washed by water for 8 hours, an ethanol-xylene series utilizing an automated tissue follow-up system. Tissues were sliced in 5 m thickness for hematoxylin-eosin (H&E) staining. Light microscope observations were made on slides (Nikon Labophot, Japan) [12].

Statistic evaluation

SPSS analyzed data (SPSS v. 26.0, IBM, Chicago, IL, USA). AST, ALT, TNF- α , and GSH-Px were represented as mean \pm SD and evaluated with one-way ANOVA ($P \leq 0.05$). Multiple comparison tests (Post Hoc Tukey HSD parametric tests) were employed to discover significant differences across groups. Histopathological status was given as median \pm SD.

RESULTS

Levels of AST, ALT, TNF- α , and GSH-Px

Compared to the sham group, the ischemia group had a substantial elevated in AST, ALT, and TNF- α ($P \leq 0.05$), and a significant lowered in GSH-Px ($P \leq 0.05$). The IP treatment of IFX and DMF lowered ALT, AST ($P \leq 0.05$), and TNF- α ($P \leq 0.05$) levels significantly. Furthermore, pretreatment significantly increased GSH-Px activity ($P \leq 0.05$). Among effective groups the combination groups more effective in lowering AST, ALT, and TNF- α ($P \leq 0.05$) than other treated groups, table (1) presents the biochemical findings of all study groups.

Table 1: Biochemical findings in all study groups (n=8) Data are presented as mean \pm SD

	Sham	I/R	I/R+IFX	I/R+DMF	I/R +Comb.
ALT (U/L)	61.38 \pm 5.63	1758.34 \pm 46.68a	854.28 \pm 10.99b	714.23 \pm 12.19c	351.28 \pm 15.69d
AST (U/L)	64.34 \pm 11.09	831.57 \pm 93.51a	433.05 \pm 44.87b	403.76 \pm 24.32c	253.32 \pm 34.07d
TNF- α (pg/mL)	40.46 \pm 4.28	427.32 \pm 28.34a	243.60 \pm 23.05b	286.42 \pm 13.25c	202.76 \pm 15.24d
GSH-Px (nmol/min/mL)	125.42 \pm 27.18	38.63 \pm 3.39a	76.51 \pm 3.05b	89.41 \pm 8.35c	99.58 \pm 11.05d
	a vs sham group $p < 0.05$	b,c vs I/R groups $p < 0.05$	d vs I/R, I/R+IFX, and I/R+DMF groups $p < 0.05$		

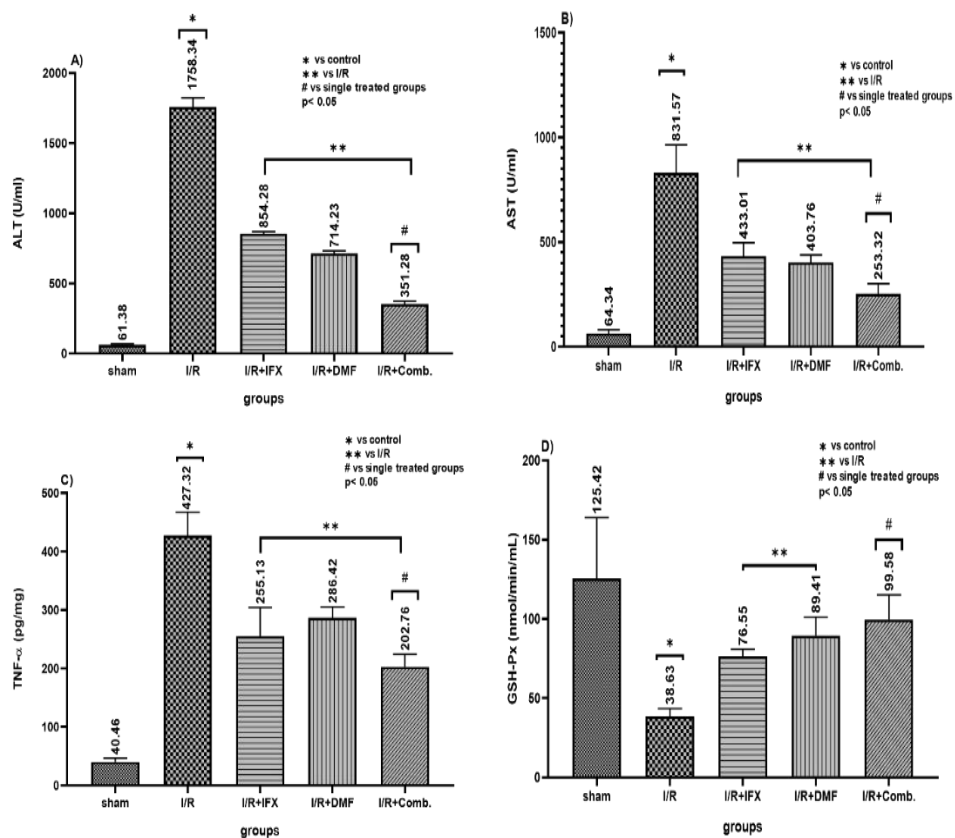


Fig. 1: Rat liver levels of an A: ALT, B: AST, C: TNF-α, and D: GSH-Px levels in response to treatment with infliximab, DMF, and their combination

The Histopathologic study

We used a score to compared between the groups according to [13] Sinusoidal dilatation, hepatocyte vacuolization and degeneration, endothelial edema, and neutrophil infiltration. When comparing the sham and I/R groups, there was increase in Sinusoidal dilatation, hepatocyte vacuolization

and degeneration, endothelial edema, and neutrophil infiltration were shown significantly (P < 0.05). In comparison to the ischemia reperfusion group, the histological evaluation of the IFX and DMF pretreated groups showed fewer histopathological alterations specifically the combination group. Table 2 and Figure 2 present the histopathologic findings.

Table 2: Different groups of rats in the study were subjected to histopathological examination.

	Sham	I/R	I/R+IFX	I/R+DMF	I/R + Comb.
Sinusoidal dilatation	0.50±0.12	3.50±0.50a	2.50±0.00 b	2.50±0.50 c	1.50±0.00 d
Hepatocyte vacuolization	0.20±0.08	4.00±0.48a	2.00±0.51 b	2.50±0.51 c	1.00±0.51 d
Hepatocyte degeneration	0.50±0.03	4.00±0.90a	1.50±0.50 b	2.00±0.50 c	1.00±0.50 d
Swelling of endothelial cells	1.00±0.52	3.50±0.50 a	2.50±0.50 b	2.00±0.00c	1.00±0.50 d
a vs sham group p<0.05		b,c vs I/R groups p<0.05		d vs I/R, I/R+IFX, and I/R+DMF groups p<0.05	

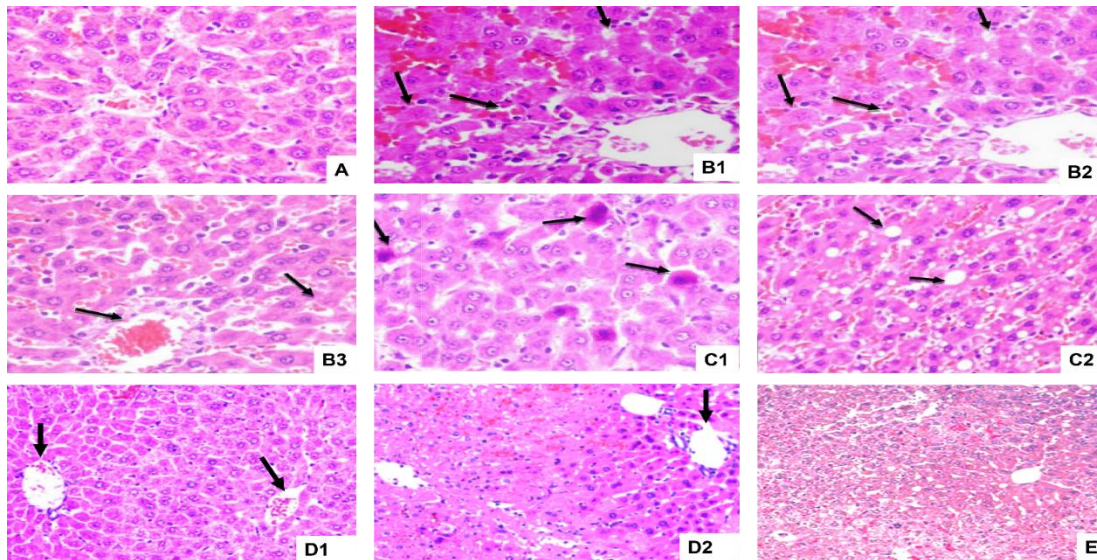


Fig. 2: Examination of the rat liver's histopathology. Sham group (A), ischemia/reperfusion (I/R) group there is a significant amount of ischemic damage (B, B1, B2, and B3), I/R+ IFX group (C1 and C2), I/R+ IFX group (D1 and D2), and I/R+ Comb. group liver sections show normal histology (E).

DISCUSSION

According to the results of our recent study, liver ischemia for half hour followed by reperfusion for one hour caused a considerable rise in ALT and AST levels. Parallel to this, a number of pathological alterations that are typical of the condition appeared, including partial hepatic parenchymal cell necrosis, bleeding, and hepatocyte edema. Many studies showed that the ROSs have a role in the pathophysiology of I/R. The antioxidant defense system is a sophisticated process that typically controls the creation of ROS. But excessive ROS production can depletion or downregulation the defense system elements [14-15]. Because they naturally contain large quantities of antioxidant enzymes including GSH-Px, superoxide dismutase, and catalase, hepatocytes are relatively resistant to ROS [16]. This study found that following I/R, ALT and AST levels as well as TNF- α level increased, most likely as a result of cell membrane injury. The pretreated groups' GSH-Px level significantly fell when compared to those in the I/R group. Furthermore, as shown by the histological analyses, I/R damage led to significant morphological alterations in the liver. These histological alterations included endothelial edema, neutrophil infiltration, sinusoidal dilatation, vacuolization and degradation of hepatocytes. The pretreatment groups clearly outperformed the I/R groups in terms of these alterations, according to a comparison between the two groups. Following the IFX-blockade of TNF- α [17] and DMF induce antioxidant system [18], which causes cellular harm by a variety of mechanisms, these benefits are thought to be related to antioxidant effects. This protective impact has been linked to its inhibition of a number of pro-apoptotic and inflammatory cytokines that were produced in response to liver injury [19-20]. The TNF- α receptors activation and oxidative stress are thought to be crucial for controlling the

inflammatory response and cell death. Furthermore, it has been stated that the phosphorylation of the NF- κ B [12, 21]. Inflammation and cellular damage were both markedly increased by the production of the NF- κ B protein. Infliximab and DMF therapy lower ROS and oxidative stress respectively by inhibiting TNF- binding to its receptor, which was dramatically reduced when both medicines were used together. Its protection against I/R-induced pathological changes in the rats' livers may be based on these therapeutic benefits.

CONCLUSIONS

Increased cytokine release and cell death signaling are the causes of hepatotoxicity. Both drugs limit cytokine release and guard against cellular damage caused by cytokine-mediated apoptosis and ROS generation pathways since infliximab is a powerful TNF- α blocker and DMF is a master oxidative stress regulator. By controlling inflammatory and apoptotic mediators, nephrotoxicity may be avoided.

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Ethical approval

KU.FVM.AEC number 0707-2022.

Funding details

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Conflict of interest

N/A

Informed Consent

the study occurred on animals with the aid of computer software

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