Original Article

DMF Attenuates Ciprofloxacin-Induced Nephropathy in Rats via Nrf2 Pathway

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ABSTRACT

Background: The pathophysiology of ciprofloxacin-induced nephropathy (CIN) is complicated by oxidative stress. The goal of this study was to see if Dimethyl Fumarate (DMF) has any antioxidant properties in a rat model of CIN.

Methods: Rats were randomly assigned to six groups (n=8): control, ciprofloxacin (ciprofloxacin-induced CIN), two DMF groups (rats treated with DMF 50mg and 100mg), and two ciprofloxacin Plus DMF groups (n=8/group) (CIN rats treated with DMF at 50 mg and 100 mg). Renal function testing, Nrf2 analysis, and anti-oxidant enzymes analysis was all done. Results: Following ciprofloxacin therapy, serum blood urea nitrogen (BUN), creatinine, and anti-oxidant enzymes all rose. In the ciprofloxacin + DMF groups, serum BUN and creatinine were lower, and anti-oxidant enzymes were higher than in the ciprofloxacin group, in CIN rats, DMF upregulated Nrf2 expression.

Conclusions: In vivo, DMF reduces experimental CIN. It's thought that this impact activates the Nrf2 antioxidant defenses pathway.

Keywords: Anti-oxidants, Ciprofloxacin, Creatinine, Nrf2, Urea.

Introduction

Oxidative stress is a harmful condition that occurs when the ratio of pro-oxidant species to anti-oxidant defense systems in a cell is out of balance, causing damage to three major macromolecules in cells: DNA, lipids, and proteins.[1] Free radical scavengers such as glutathione (GSH), ascorbic acid, and -tocopherol are examples of antioxidants. There are also detoxifying enzymes that contribute to antioxidant status, including as glutathione-S-transferase (Gsts), UDP-glucuronyl transferase (Ugts), NAD(P) H: Quinone oxidoreductase 1 (Nqo1), catalytic and modifier subunits of -glutamyl cysteine ligase (Gclc, Gclm), which synthesize GSH.[2] Drug-induced intrahepatic Cholestasis, which is common during the drug discovery and development process, could be a primary source of hepatotoxic responses.

The critical roles of reactive oxygen species (ROS) in cellular damage are well understood, and it has been postulated that the covalent binding of ROS and reactive intermediates to macromolecules may play a role in the severe adverse medication reactions.[3] Ciprofloxacin's reported hepatotoxic impact could be related to oxidative stress caused in the liver

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by the drug's creation of oxidative radicals, which leads to protein depletion in hepatocytes as a result of nucleic acid loss and DNA damage. This could result in a considerable drop in mitochondrial number and degeneration, which is responsible for energy production.[4]

Biochemical and molecular discoveries on chemical transcription factors of hepatic detoxication enzymes, as well as subsequent characterization of the modulation of drug/toxicant caused hepatotoxicity, led to the development of the NRF2 field.[5] Therefore, the activation of the Nrf2 pathway to induce the production of cytoprotective genes could be used to treat liver disorders. The purpose of the study was so that DMF could protect the liver in an acute chemical model of cipro induced hepatotoxicity. Because DMF has been shown to activate Nrf2, the renal protective effects of DMF against cipro were investigated. Rats were used to test renal toxicity.

Animals and Study Design

Forty-eight Sprague Dawley male rats will group into 6 groups. Each group contains 8 rats for a period of 30 days and Drugs preparation: Dimethyl fumarate will dissolve in DMSO and administered i.p at a dosage 50, and 100 mg/kg respectively. The animals were kept in the animal house at Faculty of Medicine, University of Kufa. The experiment was approved by the University of Kufa-Animal Care and Research Committee, and the investigation according to the Laboratory Animals Guide Care, the animals will have unrestricted access to clean water and were divided as follows:

1. The control group: given DMSO is given for 30 days.
2. The Ciprofloxacin group: given drug at a concentration of 100 mg/kg once daily by oral administration for 30 days.[6]
3. The DMF-50 group: given DMF at a concentration of 50 mg/kg once daily by IP administration for a period of 30 days.[7]
4. The DMF-100 group: given DMF at a concentration of 100 mg/kg once daily by IP administration for a period of 30 days.[8]
5. The Combination-1 group: given at ciprofloxacin 100 mg/kg once daily by oral administration for 30 days plus DMF IP with a concentration of 50 mg/kg beginning at day 10.[8,9]
6. The Combination-2 group: given at ciprofloxacin 100 mg/kg once daily by oral rout for 30 days plus DMF IP with a concentration of 100 mg/kg beginning at day 10.[8, 9]
7. At end of experiment, Animals will kill by heart puncture under ketamine 25 mg/kg and xylazine 5 to 10 mg/kg anesthesia,[10] the animals will sacrifice for collection of blood and liver tissues for further analyses.[11]

Measurement of Oxidant Parameters

According to the manufacturer's procedure and a recent study,[12] the levels of CAT, SOD, and GSH enzymes and Nrf2 were measured using two commercial detection kits (Nanjing Jincheng Bioengineering Institute).

Statistical Analysis

The results are displayed as means with standard deviation (SD). Using the SPSS software 26.0, the significance of differences in multiple group comparisons was determined using the one-way analysis of variance (ANOVA).

Results

DMF Prevents CIN- in Rats

Table 1 and Figure 1 show the serum BUN and creatinine values in each group. The serum BUN and creatinine levels in the ciprofloxacin group were considerably higher than in the control group ($P<0.05$). The DMF alone or in combination with ciprofloxacin showed lower serum levels of urea and creatinine than the ciprofloxacin group. The injection of DMF significantly reduced blood BUN and creatinine levels in the ciprofloxacin + DMF group compared to the ciprofloxacin group ($P<0.05$), indicating that DMF may have a Reno-protective effect.

DMF Attenuated oxidative stress Levels and Increased anti-oxidant enzymes Levels in Renal Tissues

DMF treatment has previously been known to improve Nrf2 and its downstream genes expression (e.g., HO-1 and NQO-1).[13-15] The administration of DMF dramatically

Table 1: Distribution of kidney makers which measure in serum among study groups

Groups/Markers	*Urea(mg/dl)*	*Creatinine (mg/dl)*	**p-value*
Control	34.50 ± 4.37	0.975±0.05	0.00
Ciprofloxacin	56.50 ± 3.16	1.98 ± 0.57	
DMF-50	35.66 ± 4.35	0.98 ± 0.38	0.96
DMF-100	34.33 ± 2.63	0.91 ± 0.58	
Combination-1	44.33 ± 3.06	1.45 ± 0.10	0.75
Combination-2	42.73 ± 2.36	1.31 ± 0.23	

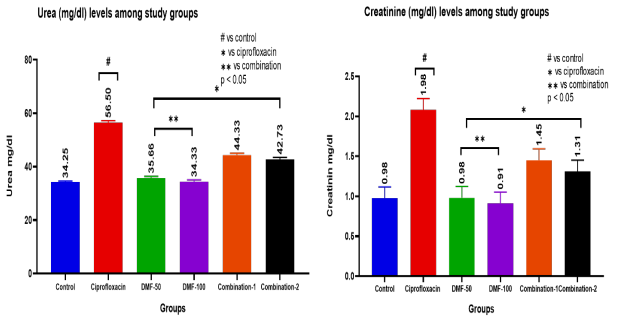

Fig.1: Represent the Urea and creatinine levels (mg/dl) among study groups

enhanced the gene expression of Nrf2 and HO-1 to see if DMF's reno-protective action in ciprofloxacin-induced nephrotoxicity is associated to Nrf2 activation. DMF significantly protected the kidney against ciprofloxacin-induced toxicity via the Nrf2 pathway, according to the findings. The antimicrobial ciprofloxacin diminished CAT, SOD, and GSH enzymes activity; however this effect was reduced by treatment with DMF. Furthermore, as showed in table 2 and Figure2, the levels of anti-oxidants enzymes in the renal tissue changed in six groups. The ciprofloxacin group's levels in renal tissue were significantly lower than the control group's ($P < 0.05$) CAT; (2.99 ± 0.35 vs. 4.82 ± 0.29), SOD; (9.97 ± 0.32 vs. 5.84 ± 0.35), and GSH; (12.37 ± 0.94 vs. 6.97 ± 0.51). In the ciprofloxacin + DMF groups, DMF significantly increased these decreases in anti-oxidant levels, while anti-oxidant levels did not differ significantly between the control and ciprofloxacin + DMF groups. In addition, there is no statistically significant difference ($p>0.05$) between the DMF groups and Combination-2 groups. There is, however, a significant increase ($p<0.05$) when we compared combination-1 groups with the ciprofloxacin and between combination-2 and combination-1.

DMF Ameliorated Renal Histological Damage

Figure 3 illustrates the pathological changes of kidney sections from all groups. The control and DMF groups' animals' kidney sections showed no significant histopathological alterations. The ciprofloxacin group's kidney sections showed significant damage, including lesions, tubular necrosis, and hemorrhagic foci. There is significantly reduced the development of these lesions and tissue damage in the ciprofloxacin + DMF group. DMF may protect CIN rats from renal histological damage, according to this kidney pathological finding.

Discussion

According to published statistics, the incidence of elevated blood creatinine and urea levels correlated with ciprofloxacin prescription ranges from 0.2 to 1.3 percent in human patients. Furthermore, ciprofloxacin-related azotemia rates have been observed to range between 1.8 and 13.1 per 1000 people treated with the antibiotic. In the lack of any differentiating clinical symptoms of nephrotoxicity other than an increase in urea and creatinine serum concentrations after administration of a fluoroquinolones, a high index of suspicion should be maintained (16). DMF has a cytoprotective effect

Table 2: Distribution of anti-oxidant enzymes among study groups

Groups/Markers	*Nrf2 ng/g of protein*	*Catalase (U/g of protein)*	*SOD (U/g of protein)*	*GSH (U/g of protein)*	**p-value*
Control	22.67 ± 0.72	4.82 ± 0.29	9.97 ± 0.32	12.37 ± 0.94	0.00
Ciprofloxacin	31.49 ± 0.43	2.99 ± 0.35	5.84 ± 0.35	6.97 ± 0.51	
DMF-50	31.66 ± 0.64	4.83 ± 0.34	9.87 ± 3.25	12.50 ± 0.47	
DMF-100	40.45 ± 1.99	4.88 ± 0.25	9.81 ± 0.12	12.92 ± 0.69	
Combination-1	41.03 ± 0.56	3.91 ± 0.25	7.26 ± 0.18	9.23 ± 0.40	
Combination-2	46.02 ± 0.50	4.53 ± 0.09	9.48 ± 0.21	11.23 ± 0.56	

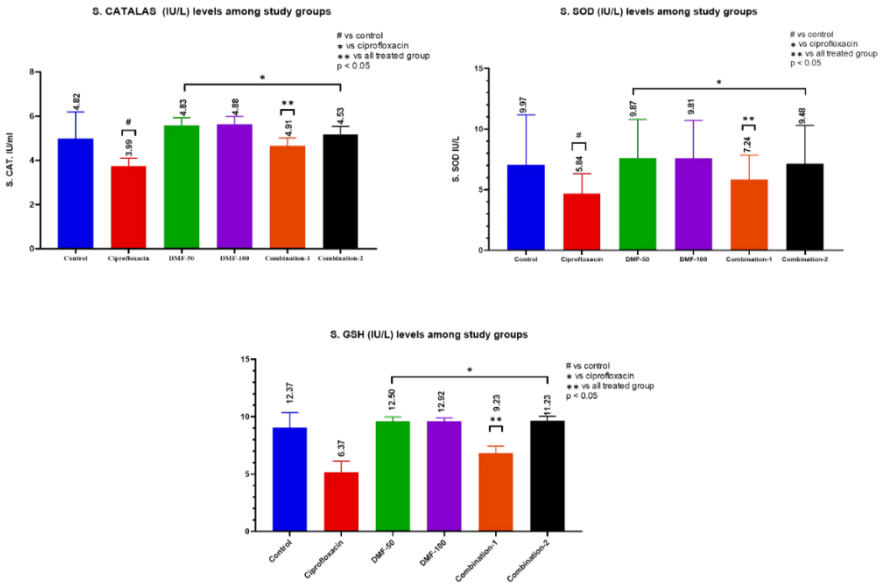

Fig. 2: Represent the anti-oxidant enzymes tissue levels (U/g of protein) among groups

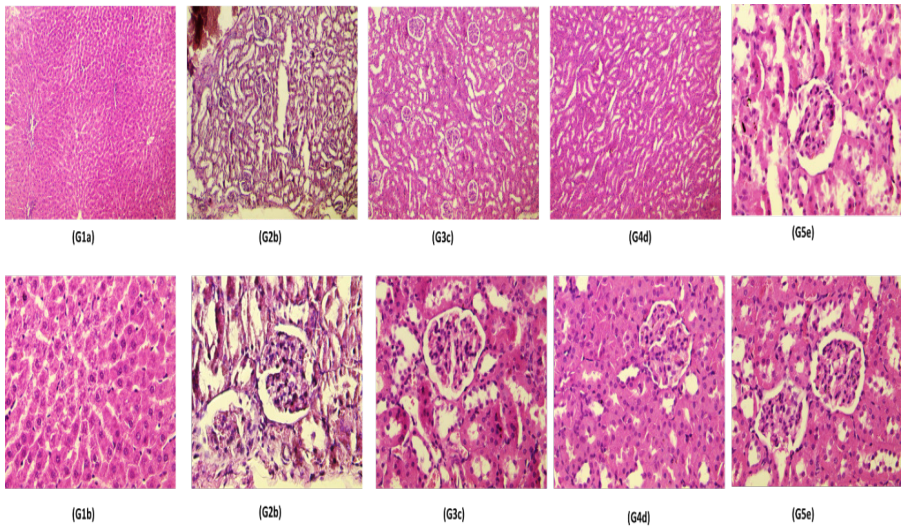

Fig.3: Representative histologic samples from several groups, control group (a1, 1b), ciprofloxacin group (b1, b2), DMF group (b1, b2) (c1, c2), and ciprofloxacin + DMF group (d1, d2) (e1, e2), Magnification: X400

of against cell injury due to nuclear Nrf2 up-regulation of and down-regulation of inflammatory and oxidative stress.[17] The administration of ciprofloxacin caused acute renal damage, according to our findings. The rats' renal function had decreased, as well as histological damage. Furthermore, the ciprofloxacin group showed significant increases in renal tissue ROS levels. In contrast, administering DMF without an inducible agent increased the anti-oxidative enzyme action by boosting the expression of the antioxidant transcription factor Nrf2, indicating that DMF has a function in renal protection. In the ciprofloxacin + DMF group, this protection seen after CIN induction dramatically reduced renal damage and decreased ROS levels. The presence of oxidative damage in the CIN rats was also revealed by the lower CAT, SOD, and GSH enzymes in the renal tissues of the ciprofloxacin group in this investigation. Treatment with DMF boosted the antioxidant enzymes activities to protect against oxidative damage in rats without CIN, as demonstrated in Figure 3. Furthermore, DMF therapy immediately scavenged ROS and increased anti-oxidant enzymes in rats with CIN. DMF's renoprotective action can be related to the elimination of excess ROS directly. Previous researches.[18-19] have reported DMF's antioxidant properties, which are consistent with our findings. In addition, DMF improved the viability of ciprofloxacin-induced injuries.

Conclusions

DMF improves CIN as assessed by renal function and kidney pathology, according to our findings. DMF significantly increased the levels of Nrf2 nuclear translation, an anti-oxidant protein. The increased antioxidant defense in the kidney and the activation of the Nrf2 pathway are primarily responsible for these positive benefits. DMF may thus be a useful medication in the prevention of CIN, although more research and randomized clinical studies are needed to confirm its preventive role.

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Ethical Approval

KU.FVM.AEC number 0706-2022.

Informed Consent

The study occurred on animals with the aid of computer software.

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