Article

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Beneficial effects of quercetin on renal injury and oxidative stress caused by ciprofloxacin in rats: A histological and biochemical study

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Abstract

Ciprofloxacin is a broad-spectrum quinolone antibiotic commonly used in clinical practice. Quercetin is an antioxidant belongs to flavonoid group. It inhibits the production of superoxide anion. In this study, we aimed to evaluate the effects of quercetin on renal injury and oxidative stress caused by ciprofloxacin. Twenty-eight female Wistar albino rats were divided into four groups: control, quercetin (20 mg kg^{-1} day^{-1} gavage for 21 days), ciprofloxacin (20 mg kg^{-1} twice a day intraperitoneally for 10 days), and ciprofloxacin + quercetin. Samples were processed for histological and biochemical evaluations. Malondialdehyde (MDA) and glutathione (GSH) levels, superoxide dismutase (SOD), and catalase (CAT) activities were measured in kidney tissue. The ciprofloxacin group showed histopathological changes such as infiltration, dilatation in tubules, tubular atrophy, reduction of Bowman's space, congestion, hemorrhage, and necrosis. In the ciprofloxacin + quercetin group, these histopathological changes markedly reduced. MDA levels increased in the ciprofloxacin group and decreased in the ciptofloxacin + quercetin group. SOD and CAT activities and GSH levels significantly decreased in the ciprofloxacin group. On the other hand, in the ciprofloxacin + quercetin group, SOD and CAT activities and GSH levels significantly increased with regard to the ciprofloxacin group. We concluded that quercetin has antioxidative and therapeutic effects on renal injury and oxidative stress caused by ciprofloxacin in rats.

Keywords

Ciprofloxacin, oxidative stress, renal injury, quercetin

Introduction

Ciprofloxacin is a synthetic broad-spectrum antimicrobial belonging to the class of fluoroquinolones.[1] It is one of the most commonly used antibiotics for different kinds of infections in the lower respiratory tract, skin, bone, joint, urinary tract, and infectious diarrhea.[2,3] Ciprofloxacin is generally well tolerated. The most common adverse reactions occur in gastrointestinal tract, central nervous system, and hematological system. Recently, rising cases of ciprofloxacin-associated organ toxicities have been reported.[4] Ciprofloxacin is mainly excreted via the kidneys.[1] Nephrotoxic reactions to fluoroquinolones appear to be uncommon but potentially serious.[5] Case reports of acute kidney injury with the use of fluoroquinolones have been published.[6]

Flavonoids are a group of naturally occurring compounds that are widely distributed as secondary metabolites in the plant kingdom. They have been recognized for their interesting clinical properties including antiinflammatory, antiallergic, antiviral, antibacterial, and antitumoral activities.[7,8] Quercetin,

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one of the most widely distributed flavonoids, has been reported to have beneficial effects to human health.[9] Quercetin scavenges free radicals directly inhibits biomolecule oxidation, prevents lipid damage, and alters antioxidant defense pathways in vivo and in vitro.[10,11]

To our knowledge, a histological study about beneficial effects of quercetin on ciprofloxacin-induced nephrotoxicity in the literature is not present. The aim of this study was to investigate the antioxidative and therapeutic effects of quercetin on ciprofloxacin-induced nephrotoxicity in Wistar albino rats. To assess the effect of quercetin, we evaluated histological and biochemical findings. We measured the levels of malondialdehyde (MDA) and glutathione (GSH), and the activities of superoxide dismutase (SOD) and catalase (CAT).

Materials and methods

Animals

Twenty-eight female Wistar albino rats (Inonu University Animal Research Center, Malatya, Turkey), weighing between 260 g and 300 g, were housed in individual cages for 21 days in a well-ventilated room with a 12-h light/12-h dark cycle at 21°C. Animals were fed with standard rat chow and tap water ad libitum. All experiments were approved by the Ethics Committee for Animal Experiments of Inonu University Faculty of Medicine and followed the National Institutes of Health's (NIH) Guide for the Care and Use of Laboratory Animals.

Groups

The rats were randomly divided into four groups and each group consisted of seven rats as follows: group 1 (control), corn oil given during 21 days by gavage; group 2 (quercetin): quercetin (20 mg kg^{-1} day^{-1}; quercetin dihydrate, 97%, CAS: 6151-25-3; Alfa Aesar, Germany) dissolved in corn oil and given during 21 day by gavage; group 3: (ciprofloxacin): ciprofloxacin (20 mg kg^{-1} twice a day; CIPRO 200 mg 100 ml^{-1} vial; Biofarma İlaç. San., Istanbul) was given intraperitoneally (ip) twice daily for 10 days (7–17 days at middle of experiment); and group 4: (ciprofloxacin + quercetin): ciprofloxacin (20 mg kg^{-1}/10 day/twice a day/ip) and quercetin (20 mg kg^{-1}/21 day/gavage) were given.

Histological evaluations

At the end of the study, the rats were sacrificed by ketamine anesthesia. The kidneys were removed by laparotomy. The tissue samples were fixed in 10% formalin solution and embedded in paraffin. The paraffin blocks were cut at 5 μm, mounted on slides, stained with hematoxylin–eosin (H-E). The sections were examined for severity of kidney injury including infiltration, dilatation of tubules, tubular atrophy, reduction of Bowman's space, congestion, hemorrhage, and tubular necrosis in 10 different fields. For this analysis, kidney injury was semiquantitatively graded as follows: 0 (*normal*), 1 (*mild*), 2 (*moderate*), and 3 (*severe*). All sections were examined using a Leica DFC280 light microscope and a Leica Q Win and Image Analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

Biochemical analysis

The remaining parts of the samples were homogenized in ice-cold 0.1 M tris(hydroxymethyl)aminomethane–hydrochloric acid buffer (pH 7.5; containing protease inhibitor, phenylmethylsulfonyl fluoride, 1 mM) with a homogenizer at 16,000 r min^{-1} for 2 min at + 4–8°C.

MDA, referred to as thiobarbituric acid reactive substances, was measured with thiobarbituric acid at 535 and 520 nm in a spectrophotometer as previously described.[12] Results were reported as nanomoles per gram wet tissue. In reduced GSH assay, GSH concentrations in the homogenates were measured according to the spectrophotometric Ellman's method.[13] Results were reported as nanomoles per gram wet tissue.

SOD activity was measured by determining the reduction of nitroblue tetrazolium (NBT) by the superoxide anion produced with xanthine and xanthine oxidase.[14] One unit of SOD was defined as the amount of protein that inhibits the rate of NBT reduction by 50% and the results reported as units per milligram protein. The specific activity of the enzymes was expressed in units per milligram (U mg^{-1}) protein. Proteins in the kidney tissue were determined by the method of Lowry et al.[15]

CAT activity was measured according to Aebi's method, by determination of the rate constant k (dimension: s^{-1}, k) of hydrogen peroxide (initial concentration 10 mM) at 240 nm in the spectrophotometer.[16] Activity was reported as k (constantrate) per milligram (k mg^{-1}) protein.

Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences for Windows Version 13.0 (SPSS Inc., Chicago, Illinois., USA) program. All data are expressed as arithmetic mean ± standard error (SE). Normality for continued variables in groups were determined by the Shapiro Wilk test. The variables didn't show normal distribution ($p < 0.05$). Kruskal–Wallis and Mann–Whitney U tests were used for comparison of variables among the studied groups. $p < 0.05$ was regarded as significant.

Results

Histological findings

Control and quercetin groups were normal in histological appearance. There was no statistically significant difference between these groups in the mean histopathological damage score (MHDS; $p > 0.05$). However, in the ciprofloxacin group, we observed histopathological changes such as peritubular infiltration, tubular dilatation, tubular atrophy, hemorrhage, and tubular necrosis. Additionally, some of the glomeruli were damaged. Congestion and reduction of Bowman's space were observed in the affected glomeruli. The MHDS was 11.42 ± 0.42 in the ciprofloxacin group. A significant increase in MHDS was detected in the ciprofloxacin group compared with the control and quercetin groups ($p < 0.001$, for both). Histopathological changes were markedly reduced in the ciprofloxacin + quercetin group. The MHDS was 6.42 ± 0.97 in this group. When ciprofloxacin and ciprofloxacin + quercetin groups were compared, statistically significant decrease was detected in MHDS ($p < 0.005$; Figure 1). The MHDS of all groups are shown in Table 1.

Biochemical findings

A significant increase was observed in mean tissue MDA levels of the ciprofloxacin group when compared with the control and quercetin group ($p < 0.005$ and $p < 0.05$, respectively). On the other hand, a significant decrease was observed in mean MDA levels of ciprofloxacin + quercetin group, when compared with the ciprofloxacin group ($p < 0.05$). Furthermore, in the ciprofloxacin group, GSH levels and SOD and CAT activities were significantly decreased when compared with the control group ($p < 0.005$, $p < 0.05$, and $p < 0.05$, respectively). On the other hand, there was a significant increase in tissue GSH levels and SOD and CAT activities in ciprofloxacin + quercetin group when compared with the ciprofloxacin group ($p < 0.05$, $p < 0.01$, and $p < 0.005$, respectively). Quercetin treatment elevated antioxidant parameters including activities of SOD and CAT and levels of GSH. Biochemical findings were consistent with histopathological findings. The mean levels of MDA and GSH and activities of SOD and CAT of all groups are shown in Table 2.

Discussion

Fluoroquinolones are most widely prescribed antibacterial agents, especially for respiratory and urinary tract infections.[17,18] Although ciprofloxacin is one of the safest and well-tolerated second-generation fluoroquinolones, in recent studies, ciprofloxacin-associated tissue damage was reported such as chondrotoxicity, testicular toxicity, nephrotoxicity, hepatotoxicity, cardiotoxicity, and neurologic symptoms.[1,2,4,17,19,20] Ciprofloxacin is not primarily nephrotoxic, but it has nephrotoxic potential especially in old age and in immunocompromised patients.[1] It is mostly associated with acute renal damage.[5,6,21] Renal dysfunction usually occurs 7–14 days after exposure, but may occur earlier in a previously sensitized individual.[21]

In our study, we found ciprofloxacin-induced histopathological changes including inflammation, dilatation in tubules, tubular atrophy, reduction of Bowman's space, congestion, hemorrhage, and necrosis in the kidney. Al-Shawi et al. also found similar histopathological changes in ciprofloxacin-induced nephrotoxicity.[22] They observed cell swelling of the epithelial lining of renal tubules and necrosis in juvenile rats. In our study, the mean histopathological score for glomerular and tubular changes was significantly higher in the ciprofloxacin group than in the control and quercetin groups. Quercetin supplementation significantly reduced these histopathological changes. To our knowledge, a light microscopic study about available effects of antioxidants on ciprofloxacin-induced nephrotoxicity in literature was found rarely. However, Gurbay et al. reported that ciprofloxacin is cytotoxic for human fibroblast cells, HeLa cells, and rat astrocytes.[23–26] They found that ciprofloxacin was showed apoptosis-inducing effect in HeLa cells.[24] Solely, they demonstrated that cytotoxic effects of ciprofloxacin were dose and time dependent.

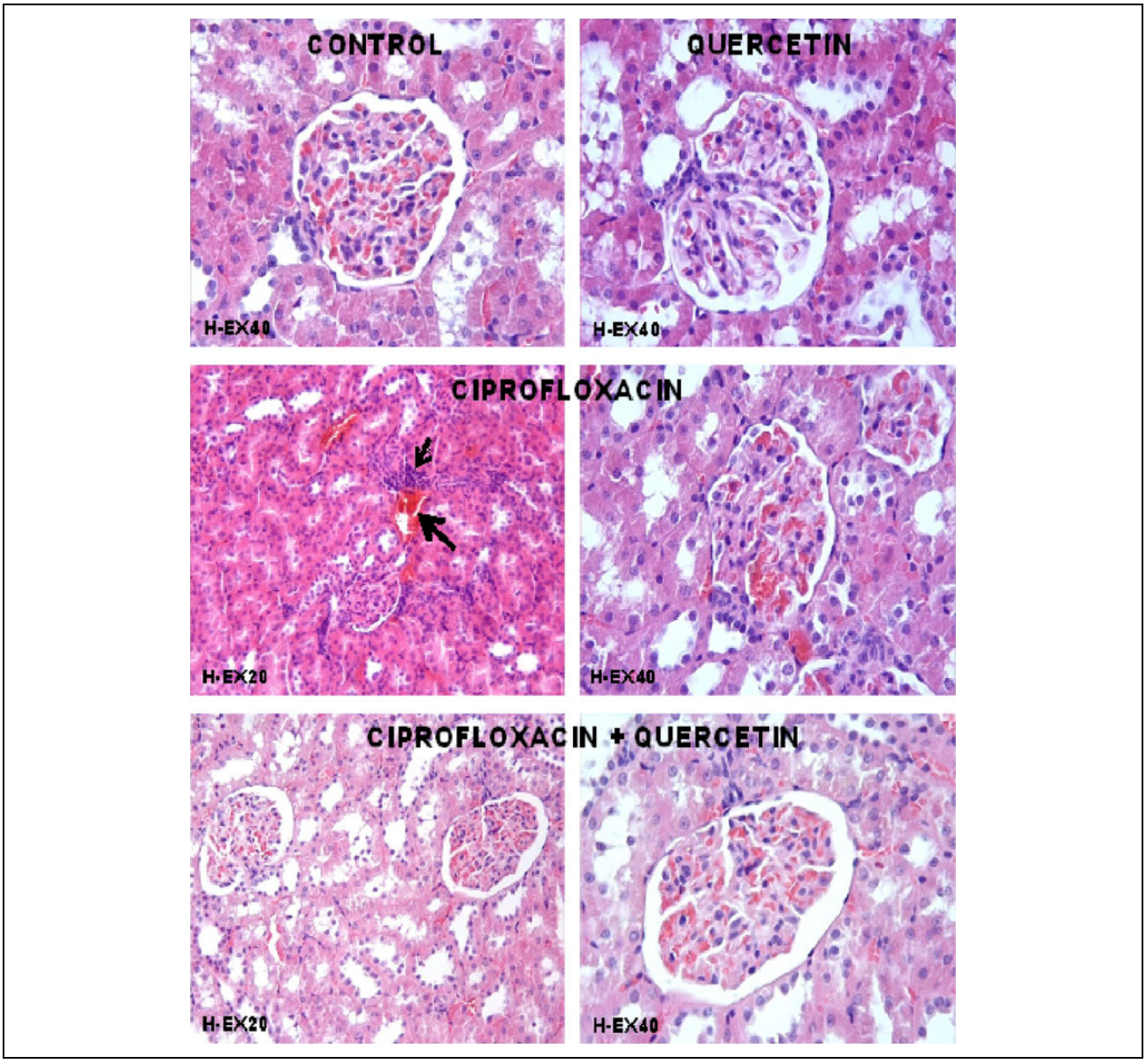

Figure 1. Control group: the glomerules and tubules are normal in histological appearance. Quercetin group: histological appearance is similar to control group. Ciprofloxacin group: prominent interstitial inflammation (thin arrow), hemorrhagic areas (thick arrow), reduction of Bowman's space, and congestion are seen. Ciprofloxacin + quercetin group: histopathological appearance of glomerules and tubules are nearly the same as those of the control group.

Table 1. The MHDS of all groups.[a]

Groups	MHDS
Group 1: control	0.00 ± 0.00
Group 2: quercetin	0.28 ± 0.18
Group 3: ciprofloxacin	11.42 ± 0.42[b]
Group 4: ciprofloxacin + quercetin	6.42 ± 0.97[c]

MHDS: mean histopathological damage score.
[a]Data are expressed arithmetic mean ± SE of seven animals.
[b]$p < 0.001$ versus group 1 and 2.
[c]$p < 0.005$ versus group 3.

Ciprofloxacin has been demonstrated to stimulate the production of reactive oxygen species (ROS) in different cells.[17,23,27,28] The formation of free radicals by ciprofloxacin may provide an explanation for the mechanism of adverse effects. The damaging effects of ROS and chemical metabolites are generally blocked by the actions of a coordinated set of antioxidant enzymes and compounds.[29]

MDA is one of the most important end products of lipid peroxidation and is known to be an indicator of free radical damage, and also indirectly of tissue

Table 2. The mean tissue oxidant–antioxidant parameters of all groups.[a]

Groups	MDA (nmol g^{-1} wet tissue)	GSH (nmol g^{-1} wet tissue)	SOD (U mg^{-1} protein)	CAT (k mg^{-1} protein)
Group 1: control	564.28 ± 34.40	893.57 ± 34.52	540.00 ± 95.83	444.85 ± 19.07
Group 2: quercetin	628.42 ± 37.41	779.28 ± 110.78	373.28 ± 23.89	480.28 ± 31.44
Group 3: ciprofloxacin	838.85 ± 26.86[b,c]	503.85 ± 61.62[b]	312.14 ± 24.58[d]	385.28 ± 14.07[d]
Group 4: ciprofloxacin + quercetin	660.28 ± 46.89[e]	753.85 ± 41.73[e]	425.71 ± 25.95[f]	463.85 ± 16.85[g]

[a]Data are expressed arithmetic mean ± SE of seven animals.
[b]$p < 0.005$ versus group 1.
[c]$p < 0.05$ versus group 2.
[d]$p < 0.05$ versus group 1.
[e]$p < 0.05$ versus group 3.
[f]$p < 0.01$ versus group 3.
[g]$p < 0.005$ versus group 3.

damage.[30] In this study, MDA levels in the ciprofloxacin group were found to be significantly higher than in the control and quercetin groups. On the other hand, in the ciprofloxacin + quercetin group, MDA levels significantly decreased. Although tissue MDA levels were clearly decreased by quercetin, its mechanism is not well understood.

The enzymatic and nonenzymatic antioxidant defenses include SOD, CAT, and GSH, which can be evaluated using easy photometric assays. These enzymes within cells remove superoxide and peroxides before they react with metal catalysis to form more reactive species.[31] In our study, ciprofloxacin administration decreased SOD and CAT activites and GSH levels in kidney tissue. The other result of the present study is that there was significant increase in the SOD and CAT activites and GSH levels in ciprofloxacin + quercetin group. Quercetin scavenges free radicals directly, inhibits biomolecule oxidation, prevents lipid damage and alters antioxidant defense pathways in vivo and in vitro.[10,11] Quercetin administration increased antioxidant enzyme activities in kidney tissue in our study. It may directly eliminate ROS or directly increase the enzyme activities.

Gurbay et al. and Hincal et al. reported that ciprofloxacin inhibited proliferation of normal human fibroblast cells via oxidative damage. They also detected that vitamin E protected these cells against the lipid peroxidation.[23,28] In another study, Gurbay et al. reported that ciprofloxacin caused dose- and time-dependent free radical production in hepatic microsomes.[32]

To our knowledge, no study about available effects of quercetin on ciprofloxacin-induced nephrotoxicity has been found in the literature. This is the first histological and biochemical study evaluating the effects of quercetin on ciprofloxacin-associated kidney damage. The present study demonstrates that administration of quercetin maintains antioxidant defenses and reduces renal oxidative damage. These effects of quercetin may be useful to preserve kidney function in ciprofloxacin toxicity.

Ciprofloxacin is a safe and useful antibacterial agent at the present time. However, we concluded that clinicians should be careful about ciprofloxacin-related renal injury. Therefore, patients' kidney function test should be obtained before ciprofloxacin clinical use.

Conflict of interest

The authors declared no conflicts of interest.

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