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Ciprofloxacin Does Not Exert Nephrotoxicity in Rats

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Abstract: Ciprofloxacin, a member of the fluoroquinolone class, is an antibiotic used as a treatment for infections including the Anthrax bacteria. Studies concerning the safety and efficacy of ciprofloxacin have been controversial with respect to nephrotoxicity. Using rats orally treated with ciprofloxacin (400mg/kg/d), the effects of this drug were analyzed measuring several indications of nephrotoxicity: β-NAG, creatinine, blood urea nitrogen, and sodium and potassium levels in blood samples. Additionally, histological analyses were performed. β-NAG values, a measure of early renal damage, were significantly increased after 4 and 7 days of treatment ($p = 0.001$ and $p = 0.004$, respectively) compared with control rats. However, 7 days post-treatment, β-NAG values decreased to control levels indicative that adaptive responses were induced to prevent nephrotoxicity. These results indicate that ciprofloxacin in doses comparable to those commonly prescribed does not induce nephrotoxicity. Altogether, they further support the idea that ciprofloxacin can be safely used.

Key words: Antibiotics, nephrotoxicity, rats

INTRODUCTION

Although antibiotics have revolutionized the management of many illnesses, they often have several side effects, one of the most important being the increased incidence of acute and chronic renal reactions[1]. The kidney is a preferential target for several environmental toxins and therapeutic agents[2-6] such as antibiotics, which interact with the kidney, especially affecting its central role in excretion, namely glomerular filtration and creatinine clearance[7,8]. Such effects could lead to acute and/or chronic renal insufficiency after long term usage[9,10]. Wide ranges of toxicity have been reported, with commonly acknowledged rates of 5% to 14% for nephrotoxicity[11]. The risk for nephrotoxicity is further increased by concurrent administration of other potential nephrotoxins, including amphotericin B, cyclosporine, diuretics, or cisplatin[12].

Ciprofloxacin, one of the newer generation of antibacterial agents, while effective in treating both gram (-) and gram (+) bacterial infections[13] has, like other antibiotics, nephrotoxic actions[14]. The excretion of ciprofloxacin by the kidneys is believed to be the cause of renal damage[15-17], however, the mechanisms of ciprofloxacin nephrotoxicity is not clear. For this reason, we decided to study this issue using a rodent model orally treated with ciprofloxacin. We evaluated N-acetyl-β-D-glucosaminidase (β-NAG), an early marker of renal damage[18,19], and several nephrotoxicity indicators: creatinine clearance, blood urea nitrogen (BUN), and sodium and potassium levels.

MATERIALS AND METHODS

Reagents: Ciprofloxacin hydrochloride was obtained from Bayer, Germany. All the other chemicals were of the highest grade of purity commercially available.

Animals and Experimental Design: Female Sprague-Dawley rats (n = 24, 180-250g), obtained from Akdeniz University Animal Laboratories (Antalya, Turkey), were maintained individually in metabolic cages and fed *ad libitum*.

Rats were divided into four groups of 6 rats each: Group one - control rats; Group two - rats treated with ciprofloxacin for 4 days; Group three - rats treated with ciprofloxacin for 7 days; and Group four - rats treated with ciprofloxacin for 7 days and then stopped for 7 days. Ciproflaxin was given as a single dose 1 day (400mg/kg/d) by nasogastric sonda.

Biochemical Determinations: Urine and blood samples were collected on 0, 4, 7 and 14 days, respectively. A certain amount of the urine samples were kept at +4°C,

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the remaining urine samples and blood were kept at -20°C. After collection of samples, all rats were sacrificed, and kidneys were removed and stored in buffered formaldehyde up to two weeks. The volume and pH of urine samples; creatinine, BUN, sodium and potassium levels in blood samples; and β-NAG and creatinine levels in urine samples were determined.

Determination of β-NAG Levels: β-NAG values were determined colorimetrically by the hydrolysis of sodium salt of its substrate 3-3' dichloro phenol sulfophthalein-N-acetyl-ß-D-glucosaminide. A standard curve of 0 to 7 U/L β-NAG was constructed. 1 ml substrate solution was pipetted into test tubes. After incubation for 5 minutes at 37°C, 0.05 ml of sample was pipetted into test tubes. Blanks, standards and samples were incubated for 15 minutes at 37°C, stop solution added, and colorimetric values determined.

Determination of BUN, Sodium, Potassium and Creatinine Levels: BUN, sodium, potassium and creatinine levels were measured in a Hitachi 911 analyzer.

Immunocytochemistry: At the end of the experiments, all rats were sacrificed, kidneys were removed, and stored in buffered formaldehyde up to two weeks, followed by paraffin embedding. After sectioning, the rat kidney morphology was analyzed for each animal, blinded to the observer, following staining with hematoxylin-eosin and visualized by light microscopy.

Statistics: Statistical significance was determined using the ANOVA test when parametric standards were present or by the Kruskall-Wallis test when parametric standards were not present. In the conditions that F value was not meaningful, Sheffe was used as the post-hoc test. For comparison of the biochemical parameters of the groups, Mann-Whitney U test was used. Significance was accepted as $p < 0.05$. Results are presented as mean ± SEM of the indicated number of experiments.

RESULTS

The excretion levels of β-NAG, as well as the levels of sodium, potassium, and BUN are shown in Table 1. No significant differences were observed for BUN ($p = 0.4$), sodium ($p = 0.5$), and potassium ($p = 0.4$) levels as well as pH ($p = 0.4$) values between control animals and animals treated for 4 days nor between the subjects treated for 7 and 14 days. Furthermore, no significant difference was observed for the creatinine clearance in all groups of animals. The weights of the rats remained unchanged.

β-NAG values increased significantly in group 2 (4 day treatment) ($p = 0.001$) and remained significantly higher after 7 days ($p=0.004$) when compared with control rats. The β-NAG values of the 4 and 7 days treated rats were not significantly different ($p = 0.06$), The values are significantly at lower levels in the 14 day treated group when compared with the 7 days treated group ($p = 0.02$) (Figure 1) returning to the same levels as control group ($p=0.8$). No pathologic symptoms were observed in kidney sections from any of the experimental animals stained with hematoxylin-eosin (not shown).

DISCUSSION

Ciprofloxacin is an antibiotic used to treat bacterial infections in many different parts of the body. This antibiotic is approved for the inhaled form of Anthrax after an individual has been exposed to the agent. However, until now, the safety and effectiveness of this compound remained unclear. We investigated the effect of orally administrated ciprofloxacin in rat kidneys in a dosage 400 mg/kg/day, in accordance with previous studies[20,21]. Our results for sodium, potassium, and BUN levels (Table 1) are in accordance with previous studies [22,23].

Table 1. Relationship between duration of therapy and concentrations of various parameters

	Parameter (SE) conc				
	Na mEq/L	K mEq/L	BUN mg/dL	Creatinine clearance mg/min	B-NAG U/L
1	134 ± 4.6	4.5 + 0.3	19.3 + 0.9	0.37 + 0.02	7.8 + 1.3
2	138 + 2.8	4.2 + 0.3	15.6 + 0.1	0.34 + 0.03	27.1 + 5.7
3	138 + 0.9	4.6 + 0.2	15.5 + 0.9	0.40 + 0.06	15.6 + 2.1
4	135 + 1.2	4.2 + 0.3	16.1 + 0.6	0.10 + 0.02	7.5 + 1.2

Although the creatinine clearance showed slight variation in rats treated with ciprofloxacin, these differences were not significant when compared with control animals ($p > 0.05$). Basaran et al.[23] observed an increase in creatinine clearance during ciprofloxacin treatment. The different results obtained are most likely due to differences in the methodology used. However, similarly to our results, they did not find histological changes in the kidney. Furthermore, Falkenberg and Mondorf[24] found no tubular damage in their ciprofloxacin nephrotoxicity study.

To determine renal deterioration at early stages, β-NAG values were analyzed and it was observed that ciprofloxacin therapy alters β-NAG concentrations (Table 1; Figure 1), which indicates that β-NAG is a suitable

parameter for the evaluation of renal function damage. Importantly, it was observed that β-NAG values return to normal after the treatment is stopped (Table 1). Yazaki et al.[25] observed that isepamicin, a broad spectrum aminoglycoside, increased NAG, BUN and serum creatinine. However, these effects could be prevented by fleroxacin, a broad-range antimicrobial spectrum.

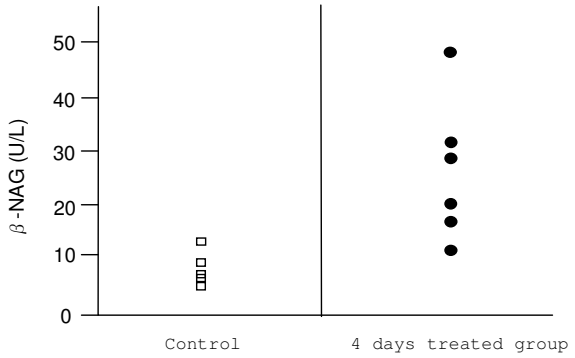

Fig. 1: ß-NAG values of the 4 day treatment group.

β-NAG values were highest in the 4 day treated animals and were lower in the 7 day treated rats. Similarly, William and Kenneth[17] reported improvement in the clinical symptoms of nephrotoxicity in patients after ciprofloxacin treatment was stopped. The fact that the levels of β-NAG in group four is similar to that of control animals suggests that the animals develop adaptive responses that lead to the normalization of β-NAG values.

Overall, we did not observe any dose-dependent toxicity induced by ciprofloxacin, although previous studies reported a dose-dependent toxicity[26]. Altogether, our data suggests that ciprofloxacin does not have any long-term effect on kidney function or morphology, despite the higher levels of β-NAG observed during the treatment. The dosage of used in this study falls in the range given to human subjects (for more information, see http://www.nlm.nih.gov/medlineplus/druginfo/uspdi/202656.html). In conclusion, despite the increased levels of β-NAG in early stages of drug administration, ciprofloxacin does not exert nephrotoxicity.

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