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Ciprofloxacin increases hepatic and renal lipid hydroperoxides levels in mice

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ABSTRACT: Ciprofloxacin (CFX) is an effective and relatively safe antimicrobial used in a variety of human infections. However, adverse drug reactions and positive results in genotoxic tests are reported.

In order to understand the possible pathophysiological mechanisms of the toxic effects informed for CFX, lipid hydroperoxides (LOOH) -oxidative mediators of peroxidation- were quantified in liver and kidney of mice, after 15 to 360 minutes of the ciprofloxacin administration at doses of 10 mg/ Kg or 100 mg/ Kg by ip route. The peroxidation in the lipid fraction was evaluated by measuring the amount of hydroperoxides through the oxidation of 1- naphthyldiphenylphospine into its oxide and further quantification by high performance liquid chromatography.

The initial content of lipid hydroperoxides (nmol/g tissue) was 253 ± 3 in kidney and 143 ± 12 in liver. CFX induced the maximal variation to 728 ± 101 in kidney ($P < 0.05$) and 315 ± 31 in liver ($P < 0.01$), after 15 min of 100 mg/ Kg single dose. The variation in the LOOH levels was significant in kidney with both doses used and in liver after 100 mg/ Kg until 60 min after the CFX administration, and then gradually fell to natural levels.

The results demonstrated the effect of CFX on lipid oxidation, an indicator of oxidative effect. A natural protective capacity against this oxidation, more efficient in liver than in kidney, was observed.

Introduction

Ciprofloxacin (CFX) is a fluoroquinolone of second generation with a broad spectrum of antibacterial activity. This drug has good bioavailability after oral administration, good to excellent tissue penetration and relative safety (Ball and Tillotson, 1995; Papich, 1998). For these reasons, it is used in a variety of human clinical infections (Suh and Lorber, 1995), particularly urinary in a media dose of 10 mg/ kg/ day, during 1 to 12 weeks.

One of the most important concern is its arthropathogenic potential, derived mainly from arthropathies and irreversible erosions observed in juvenile animals (dog, rat, rabbit) (Roger, 1996). Human rheumatologic alterations are rare and consist of myalgia, arthralgia and arthritis (Zabraniecki *et al.*, 1996). The pathophysiology of the adverse drug reactions by CFX is not well known. Quinolones induce *in vitro* an early stimulation of the oxidative metabolism in immature rabbit condrocytes (Thoung Guyot *et al.*, 1996) and differential effect on newly differentiating *in vitro* human cells (Mont *et al.*, 1996). Genotoxicity *in vitro* studies have reported mutagenicity in bacteria (Majtanova and Majtan, 1996), and in mouse lymphoma cells (Chetelat *et al.*, 1996). Positive results were also observed in cytogenetic studies *in vitro* and *in vivo*, unscheduled DNA synthesis and alkaline elution tests (Domagala, 1994; Takayama *et al.*, 1995; Gorla *et al.*, 1999).

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The formation of reactive oxygen species appears to be responsible for the ability of fluoroquinoles to cause light-induced adverse drug effects, and oxygen radical scavengers modulate the photoclastogenicity and phototoxicity reported (Chetelat *et al*., 1996). Lipid hydroperoxides (LOOH) are oxidative mediators of lipid peroxidation and relevant works have associated LOOH with serious pathological conditions of oxidative mechanisms and induced tissue damage (Cheeseman, 1993; Coyle and Puttfarcken, 1993; Díaz Velez *et al*., 1996). In this work, LOOH formation was quantified through the oxidation of 1- naphthyldiphenylphospine (NDPP) into its oxide, in liver and kidney of mice administered with a single dose, in order to understand the possible pathophysiological mechanisms of the toxic effects informed for CFX.

Materials and methods

Animals and treatment

Male and female Balb C mice of 8-12 weeks old (n= 48) were used in the experiments. Animals were distributed into two control groups without treatment, seven groups of three animals each one for 10 mg/ Kg ciprofloxacin single dose and other seven for 100 mg/ Kg administration. Ciprofloxacin (Parafarm) in 0.5 ml/ 25 g body weight was injected intraperitoneally and mice sacrified at 15, 30, 45, 60, 120, 240 and 360 min after antimicrobial treatment. Liver and kidneys were processed as indicated (Weyers *et al*., 2001). After tissue homogenization, the lipids were twice extracted with chloroform/ methanol (2:1, v/v) containing 0.03% of butylated hydroxytoluene. The organic layers were evaporated to dryness and the lipid extract was dissolved in 300 ul chloroform: methanol (1: 2, v:v) for LOOH determination.

Analysis of lipid hydroperoxide levels

Lipid hydroperoxide levels were evaluated through the reaction with NDPP into 1- naphthyldiphenylphospine oxide (ONDPP) in a black tube at 60ºC, during 60 min (Tokumaru *et al*., 1995). ONDPP generated stoichiometrically was quantified on a Hewlett Packard high performance liquid chromatographic system, reverse-phase ODS (C_{18}) column at 292 nm. The mobile phase was methanol: water (90: 10) and the flow rate 1.0 mL/ min. Results were expressed as nmols of LOOH/ g of tissue. Hydroperoxide levels were calculated using t-butyl hydroperoxide as a standard with a linear detection from 0.0312 to 1.0 mM and a coefficient of correlation of 0.991.

Statistical analysis

Welch's t-test has been performed for the differences in LOOH levels, between controls and after CFX administered groups.

TABLE 1.

Minutes after ciprofloxacin administration	Liver (nmols of LOOH/ g tissue)		Kidney (nmols of LOOH/ g tissue)	
	10 mg/ Kg	100 mg/ Kg	10 mg/ Kg	100 mg/ Kg
Control	134 ± 15	143 ± 12	282 ± 15	253 ± 3
15	169 ± 29	315** ± 31	536* ± 82	728* ± 101
30	137 ± 11	217** ± 17	368* ± 24	377 ± 62
45	148 ± 10	214*** ± 2	529* ± 51	468** ± 33
60	151 ± 9	207 ***± 9	474* ± 28	486* ± 58
120	100 ± 25	94 ± 25	300 ± 35	271 ± 20
240	91 ± 16	87 ± 20	281 ± 17	317 ± 42
360	140 ± 39	91 ± 5	314 ± 49	299 ± 26

Lipid hydroperoxide (LOOH) levels in kidney and liver of mice after 10 or 100 mg/ Kg ciprofloxacin single dose. Each data is mean ± SEM from 3 mice, P (Welch´s t test) between control and administered. * P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001.

Results

Lipid hydroperoxide levels in liver and kidney of mice at 15, 30, 45, 60, 120, 240 and 360 min after a single CFX administration (10 and 100 mg/ Kg), expressed in nmols of LOOH/ g of tissue are shown in Table 1. Control levels of LOOH in kidney were 253 $\pm$ 3 nmol of LOOH/ g tissue, and in liver were 143 $\pm$ 12 nmol of LOOH/ g tissue.

CFX induced a variation in the natural levels of LOOH. The variation was maximal at 100 mg/ Kg of CFX and after 15 min of the administration. From their initial levels CFX induced an increase to 728 $\pm$ 101 ($P < 0.05$) in kidney and 315 $\pm$ 31 ($P < 0.01$) in liver, after 15 min of the antimicrobial administration. From this point, levels slowly descended, being at 60 min, 486 $\pm$ 58 for kidney and 207 $\pm$ 9 for liver, $P < 0.05$ and $P < 0.001$ respectively, still significant when compared to control values. At 10 mg/ Kg and 100 mg/ Kg, the increase observed in the LOOH levels after 15 min was major in kidney (90% - 188%) than in liver (27% - 120%).

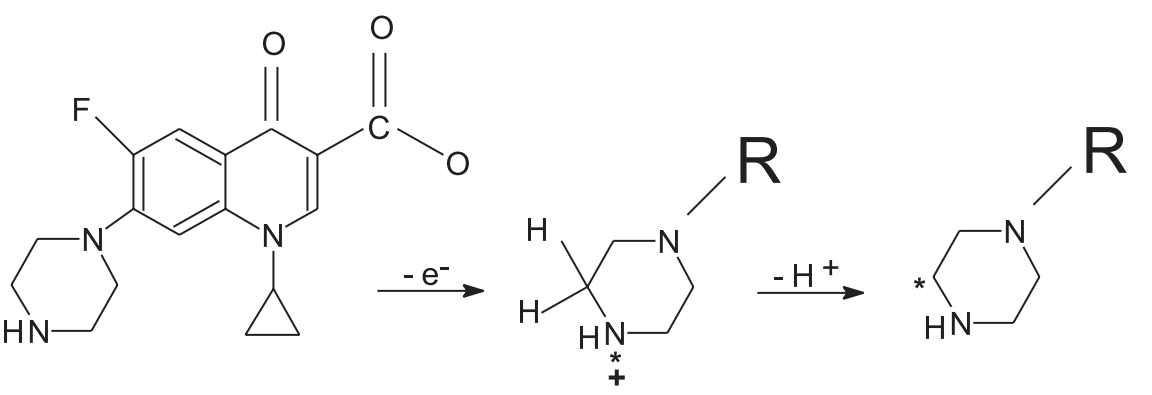
The last determination was performed at 360 min with LOOH levels in kidney and liver similar to the initial ones at 10 mg/ Kg, and in liver significantly below the initial values at 100 mg/ Kg (Table 1). The reaction of kidney and liver induced by CFX was different with both doses. In liver CFX did not induce significant changes at 10 mg/ Kg and variations were observed from 15 to 60 min after 100 mg/ Kg CFX administration. In the kidney, the change in the LOOH level was significantly different from the control level up to 60 min at 10 mg and 100 mg/ Kg.

Discussion

LOOH levels in liver and kidney of mice induced by CFX were detected by the NDPP method. NDPP is oxidized to ONDPP through the quantitative and stoichiometric reduction of lipid hydroperoxides. Tokumaru´s original assay (1995) for LOOH determination in tissues has proven to be sensitive and reliable for the determination of oxidative stress in mammalian organs (Weyers *et al*., 2001). Control levels of LOOH in kidney were higher than those in liver. This was observed in a previous work (Weyers *et al*., 2001) and by other authors in 85 weeks old mice (Tokumaru *et al*., 1996).

The variation in the LOOH levels was significant in kidney after both doses used and in liver after 100 mg/ Kg until 60 min after the CFX administration, and then gradually fell to natural levels. It is well known that firstly liver, and also kidney are the major organs of biotransformation in animals. The observed oxidative effect of CFX was more important in kidney than in liver. In both organs, the antioxidative cellular system reverts to normal concentrations the variation of LOOH induced by CFX. The natural protective capacity was more efficient in liver than in kidney evidenced in the minor absolute physiological and induced levels of LOOH. At 100 mg/ Kg in liver, levels of LOOH descended below the control values, due to the protective capacity of the liver (Table 1).

After the CFX administration, the maximal concentration of LOOH at 15 min, clearly demonstrated the early effect of CFX on lipid oxidation. This variation in the LOOH levels can be explained as a consequence of the possible metabolic transformation of CFX. This antimicrobial is a 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3quinolone carboxylic acid and its extensive metabolism seems to be centered in the piperazynil ring (Nouws *et al*., 1998). The ring may become oxidized to 4-oxo CFX or opened to desethylen CFX (Nouws *et al*., 1998; Kamberri *et al*., 1998). To our opinion at least two free radical intermediates may be generated, beginning with one of the nitrogens of the piperazinyl ring of ciprofloxacin:



A first intermediate radical with an unpaired electron(*), and hence extremely reactive, could generate other in the contiguous carbon to yield a secondary intermediate radical. It could react with lipids in a cascade of peroxidative reactions which could explain the increase of LOOH levels induced by CFX in this study.

LOOH formation is oxidative indicator of lipid peroxidation and in this way it could participate in the pathophysiological mechanisms of CFX toxicity. The oxidative stress, induced by CFX, was also reported in cultured articular condrocytes (Thoung Guyot *et al*., 1996).

In this work a transitory increase of lipid hydroperoxides was observed, reverted by cellular mechanisms. Arthropathy toxicity by CFX reported in the literature is produced in juvenile rats after weeks of treatment with quinolones (Forster *et al*., 1997). Then, it would be important to investigate if the antioxidative cellular systems can also prevent the variations in the LOOH levels in a common therapeutic schedule (1- 12 weeks of treatment).

LOOH variations were dose dependent in both organs, and kidney has shown to be more sensitive than liver to the oxidative effect. CFX can disturb the delicate balance between intracellular pro-oxidant and antioxidant homeostasis and this could be kept on mind in the inter-individual variability of the adverse effects to this antimicrobial as a possible mechanism of toxicity.

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References

BALL P, TILLOTSON G (1995). Tolerability of Fluoroquinole antibiotics Past, Present and Future. Drug safety 13: 345-358.

CHEESEMAN KH (1993). Lipid peroxidation and cancer. In: Halliwell B, Aruoma OI eds. DNA and free radicals, New York: Ellis Horwood; 109-144.

CHETELAT A, ALBERTINI S, GOCKE E (1996). The photomutagenicity of fluoroquinolones in tests for gene mutation, chromosomal aberration, gene convertion and DNA breakage (Comet assay). Mutagenesis 11: 497-504.

COYLE JT, PUTTFARCKEN P (1993). Oxidative stress, glutamate, and neurodegenerative disorders. Science 262: 680-695.

DÍAZ VELEZ CR, GARCÍA CASTAÑEIRAS S, MENDOZA RAMOS E, HERNANDEZ LOPEZ E (1996). Increased malondialdehyde in peripheral blood of patients with congestive heart failure. Am Heart J 131: 146-152.

DOMAGALA J (1994). Structure activity and structure side effects relationships for the quinolone antibacterials. J Antim Chemother 33: 685-706.

FORSTER C, SCHWABE R, LOZO E, ZIPPEL U, VORMANN J, GUNTHER T, MERKER HJ, STAHLMANN (1997). Quinolone induced arthropathy: exposure of magnesium deficient aged rats or inmature rats, mineral concentrations in target tissues and pharmacokinetics. Arch Toxicol 72: 26-32.

GORLA N, GARCÍA OVANDO H, LARRIPA I (1999). Chromosomal aberrations in human lymphocytes exposed in vitro to enrofloxacin and ciprofloxacin. Toxicol Letters 104: 43-48.

KAMBERRI M, TSUTSUMI K, KOTEGAWA T, NAKAMURA K, NAKANO S (1998). Determination of ciprofloxacin in plasma and urine by HPLC with ultraviolet detection. Clin Chem 44: 6, 1251-1255.

MAJTANOVA L, MAJTAN V (1996). Quinolone effects in the SOS Chromotest and in the synthesis of biomacromolecules. Folia Microbiol 41: 233-236.

MONT M, MATHUR S, FRONDOZA G, HUNGERFORD D (1996). The effects of ciprofloxacin on human chondrocytes in cell culture. Infection 24: 151-155.

NOUWS JFM, MEVIUS DJ, VREE TB, BAARS AM LAURENSEN J (1998) Pharmacokinetics, renal clearance and metabolism of ciprofloxacin following intravenous and oral administartion to calves. Vet Quaterly 10: 156-163.

PAPICH M (1998). Antibacterial drug Therapy Focus on new drugs. Clin Pharmacol Therap 28: 215-231.

ROYER R (1996). Adverse drug reactions with fluoroquinolones. Therapie 51: 414-416.

SUH B, LORBER B (1995). Quinolones. Med Clin NA 79: 869-894.

TAKAYAMA S, HIROHASHI M, KATO M, SHIMADA H (1995). Toxicity of quinolone antimicrobial agents. J Tox Environ Health 45: 1-45.

THOUNG GUYOT M, DOMARLE O, POCIDALO J, HAYEM G (1996). Effects of fluoroquinolones on cultured articular chondrocytes flow cytometric analysis of free radical production. J Pharmacol Exp Therap 271: 1544-1549.

TOKUMARU S, IGUCHI H, KOJO S (1996). Change of the lipid hydroperoxide level in mouse organs on ageing. Mechanisms of Ageing and Development 86: 67-74.

TOKUMARU S, TSUKAMOTO I, IGUCHI H, KOJO S (1995). Specific and sensitive determination of lipid hydroperoxides with chemical derivatization into 1-naphthyldiphenylphosphine oxide and high-performance liquid chromatography. Anal Chim Acta 307: 7-10.

WEYERS A, GORLA N, UGNIA L, GARCÍA OVANDO H, CHESTA C (2001). Lipid hydroperoxides levels in liver and kidney of mice before and after Cl_4C single administration. Biocell 25: 11-15.

ZABRANIECKI L, NEGRIER I, VERGNE P, ARNAUD M, BONNET C, BERTIN P, TREVES R (1996). Fluoroquinolones induced tendinopathy: report of 6 cases. J Rheumatol 23: 516-520.