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Systemic Exposure, Tissue Distribution, and Disease Evolution of a High Solubility Ciprofloxacin−Aluminum Complex in a Murine Model of Septicemia Induced by *Salmonella enterica* Serotype Enteritidis

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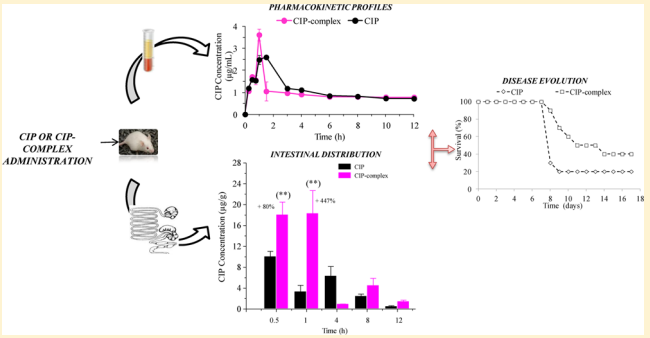
ABSTRACT: A new pharmaceutical derivative obtained by stoichiometric complexation of ciprofloxacin (CIP) with aluminum (CIP-complex) has been investigated and reported in this study. Such product has high solubility in the gastrointestinal pH range and was successful in the development of optimized formulations while maintaining its antimicrobial potency. The systemic exposure, tissue distribution, and the disease evolution after given CIP-complex were assessed. The systemic exposure and distribution in intestines, lungs, and kidneys after a single intragastric administration of CIP-complex and CIP given alone, used as reference, were performed in Balb-C mice at a dose of 5 mg CIP/kg. For the assessment of the disease evolution assay, mice were infected with a virulent strain of *Salmonella enterica* serotype Enteritidis and treated intragastrically once or twice daily during 5 consecutive days with solutions of CIP-complex or the reference. Clinical follow up and survival was measured during 15 days post inoculation and health state was scored during this period from 0 to 5. CIP-complex showed a 32% increase in C_{max}, an earlier T_{max}, and a smaller AUC_{0-12} than the reference. Maximum tissue concentrations (0.5−1 h) were significantly higher in CIP-complex (447% in intestine, 93% in kidney, and 44% in lungs). In the infection model used in this study, survival in CIP-complex versus CIP groups was 40% versus 20% (twice-daily administration) and 30% versus 0% (once-daily administration). Health state of the survivors of CIP-complex group (5/5) was higher than CIP group (3/5). The greater effectiveness of CIP-complex is attributed to the higher levels of CIP in the intestine. Our results supported the fact that CIP-complex is a promising candidate to develop dose-efficient formulations of CIP for oral administration.



KEYWORDS: *fluoroquinolone complexes, infection model, pharmacokinetics, pharmacodynamics, disease evolution, biopharmaceutic classification system*

INTRODUCTION

Ciprofloxacin (CIP, Figure 1a), a synthetic fluorinated 4-quinolone has a broad spectrum antimicrobial activity. According to WHO model list of essential medicines,[1] CIP, as the hydrochloride salt, is considered an essential drug for the treatment of different infectious diseases. Because of its chemical structure, CIP is a zwitterion and exhibits a U-shaped pH solubility profile, with high solubility at pH values below 5 and above 10, and minimum solubility near the isoelectric point, which is close to neutral. CIP hydrochloride is a class IV drug substance, according to the Biopharmaceutic Classification System (low solubility related to dose and low intestinal permeability).[2] Its low solubility at pHs embracing gastrointestinal tract may lead to incomplete in vivo dissolution after oral administration.[3] The bioavailability of CIP has been extensively studied. Absolute bioavailability of CIP after oral administration is low and erratic (56−77%).[4−6] With oral administration, a noticeable trend in the increasing half-life with increasing dose from 250 to 1000 mg was observed and a slow continuous absorption phase due to pH-dependent phenomena was suggested as a possible explanation.[7] Besides, a reduction of the fraction absorbed and a later elimination, assessed by urinary recovery data, was observed as the oral dose of CIP

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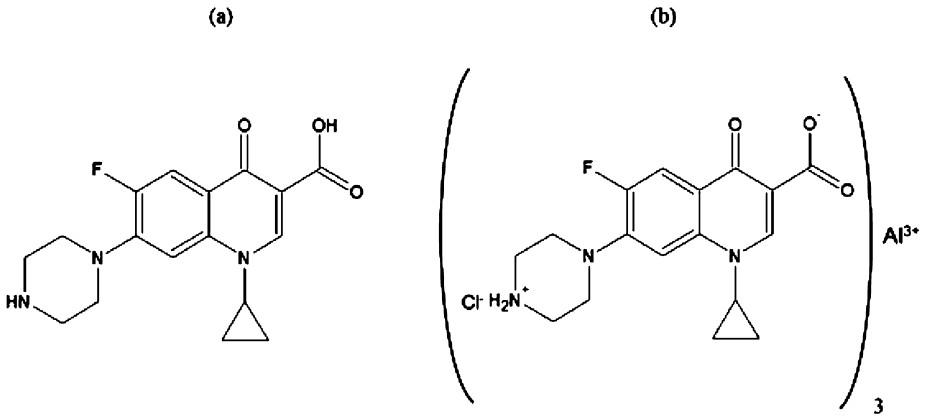

Figure 1. Chemical structure of (a) ciprofloxacin, CIP, and (b) hydrochloride of the aluminum complex of ciprofloxacin, CIP-complex.

increases.[7,8] In agreement, T_{max} is significantly longer with the 750 mg dose than with the 200 mg dose (1.38 h versus 0.69 h),[6−8] showing that the events leading up to the absorption step were rate limited.[7,8] CIP low solubility concerns are not limited to oral administration. In ophthalmic formulations containing CIP hydrochloride (pH range of the solution 3.5−5.5),[9] the precipitation of CIP has been quantitatively demonstrated to be driven by supersaturation as pH and drug concentration normalizes following ocular instillation.[10] In fact, topical administration of CIP has been associated with corneal deposits.[11−13] CIP's low solubility has also limited the development of suitable parenteral dosage forms, and hence, only large volume parenteral solutions (i.e., 400 mg of CIP in 200 mL solution) for intravenous infusion are currently available, which are stated to have an acidic pH of 3.9−4.5 needed to maintain the dose in solution.[14] These formulations therefore require a slow administration rate to minimize discomfort and reduce the risk of venous irritation.[15]

Thus, novel strategies for improving CIP performance are needed. The pharmaceutical industry typically employs several methods for correcting compounds that exhibit undesirable physicochemical characteristics including screening for salts, complexes, polymorphs, and hydrates/solvates of the active pharmaceutical ingredient. These kinds of substances are interesting since they can be used to both modify the physicochemical properties of a compound without affecting the intrinsic bioactivity and extend the product life of the active pharmaceutical ingredient.[16] Whenever solubility is a limiting factor in the bioavailability of a compound, modulation of solubility may produce drastic effects.

To overcome solubility concerns of CIP, the hydrochloride of its aluminum complex (CIP-complex, Figure 1b) was obtained and thoroughly characterized as a new pharmaceutical derivative of CIP.[17,18] The biological and physicochemical properties of pharmaceutical interest of CIP-complex have already been reported. CIP-complex is obtained as a pure and stable crystalline solid[18,19] with a definite stoichiometric composition of 3:1 CIP/aluminum and presents high aqueous solubility and fast dissolution rate.[3] CIP-complex can be synthesized by two different methods. One of them will allow obtaining the compound at the production scale.[19] The antibiotic potency of CIP-complex showed no differences with the uncomplexed form.[20] Upon aluminum complexation, the solubility increases 20 times at pH 5.6 and 37 °C. Although a reduction in solubility is observed as the pH increases, the solubility of CIP-complex at intestinal pH 6.9 is still 14 times higher than CIP.[3] Taking advantage of the solubility improvement, clear solutions of 0.5 and 1% of CIP, with a pH of 7.2 were prepared with CIP-complex to be used as ophthalmic formulations. The solutions exhibited good physical, chemical, and antimicrobial activity and satisfactorily overcame an ocular test on rabbits.[21] The increased solubility observed in glycerin allows to obtain an optimized 0.3% ototopic formulation when CIP-complex is employed. This formulation was assayed in vivo in a controlled clinical trial for the treatment of acute external otitis to evaluate efficacy and tolerability using an equimolar conventional aqueous formulation of CIP hydrochloride as reference. It was observed that discharge, swelling, pain, and redness are resolved more quickly in the CIP-complex group. Both treatments were also equally well tolerated.[22]

The high solubility in biorelevant media also sets CIP-complex as a class III drug according to the Biopharmaceutic Classification System (high solubility related to dose and low intestinal permeability) and turned it as a potential candidate for biowaiver from the scientific point of view.[3] Besides, more dose-efficient formulations for oral administration were developed, which allow keeping the complete dose in solution in the entire intestinal pH range, increasing the offer of drug available for absorption.

The antimicrobial therapy is not needed in the majority of *Salmonella* cases and states under which circumstances salmonellosis requires antimicrobial therapy. CIP has excellent in vitro activity against *Salmonella* species and is used in the treatment of *Salmonella* infections worldwide.[1,14,23−26]

The main goal of the present study was to assess the systemic exposure, tissue distribution, and consequent efficacy of the novel CIP-complex in comparison with CIP, against a septic murine model using *Salmonella enterica* serotype Enteritidis as a lethal pathogen.

EXPERIMENTAL SECTION

Reagents and Solvents. CIP-complex was obtained according to the procedure of AR007762B1 patent. CIP hydrochloride (analytical grade, Parafarm, Argentina) and aluminum chloride-6-hydrate extra pure (Ph. Eur, Riedel-de Haehn, Germany) were used. Danofloxacin (DAN), Marbofloxacin (MAR), and triethylamine were analytical grade and purchased from Sigma (Sigma Aldrich srl, EE.UU). Phosphoric acid (85%) was provided by Baker (EE.UU). Acetonitrile and methanol were HPLC grade solvent from Sintorgran (Argentina). Double distilled and deionized water (Milli-Q water) was obtained using a commercial water purification

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system (Simplicity, Millipore, Brazil). Phosphate buffer pH 7.0 was prepared according to USP 33-NF 28, dissolving 3.54 g of potassium dihydrogen phosphate (analytical grade, Baker, EEUU) and 5.82 g of anhydrous sodium dihydrogen phosphate (analytical grade, Baker, EE.UU) in 1000 mL of Milli-Q water and then adjusting the pH with 1 N NaOH (analytical grade Anedra, Argentina).

Equipment and Chromatographic Conditions. The chromatographic conditions reported by Gonzalez were used.[29] A Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan), comprising an LC-10AS liquid chromatograph with an RF-10A spectrofluorometric detector, a CTO-10A VP column oven, an Auto Inyector Shimadzu SIL-10 AD VP, and a Communications Bus Module-101, was used. Data were collected and analyzed using the Shimadzu Class LC10 software (SPD-10A) package. A Luna 5 μm particle size, 250 mm × 4.6 mm C_{18} reversed-phase column (Phenomenex Torrance, CA, USA) and guard column (Phenomenex) were used for separation.

For plasma analysis, the mobile phase consisted of 16% acetonitrile/methanol (13:1) mixture and 84% water containing 0.4% triethylamine and 0.4% phosphoric acid (85%) to adjust to pH 2.5. For elution of tissue extracts, the composition of acetonitrile in the mobile phase was slightly modified (14%) and the methanol omitted. The flow rate was 1.2 mL/min. All analytes were detected by fluorescence at excitation and emission wavelengths of 294 and 500 nm, respectively.

Animals. Pathogen-free adult Balb-C mice (body weight of approximately 25 g) were used. The experiments were approved by the Ethic Committee of the Faculty of Veterinary Medicine of UNCPBA-Tandil (www.vet.unicen.edu.ar). The animal experiments were performed in accordance with the standards of international good practices for animals.[27,28] Mice were obtained from the Laboratory of Pharmacology, Faculty of Veterinary Medicine, and were housed under standard laboratory conditions with free access to food and water ad libitum.

Antimicrobial Solutions. CIP-complex solutions were prepared by dissolving in water. Reference solutions of CIP were prepared by dissolving CIP hydrochloride, at the same concentration, in water.

Both CIP-complex and reference solutions had a concentration of CIP equivalent to 1.2 mM and contained a dose of 5 mg CIP/kg in a volume of 300 μL. The solutions were stored under light protecting conditions and used for the assessment of plasma drug concentrations, tissue distribution, and efficacy tests.

Systemic Exposure and Tissue Distribution Studies. For the comparative study, 88 mice were randomly divided into two treatment groups (n = 44). All experimental animals received intragastrically a single dose (300 μL) of CIP-complex or reference solutions.

After treatments, 4 mice per group were humanly sacrificed at each time point post-administration 0; 0.5; 0.75; 1; 1.5; 3; 4; 6; 8; 10; and 12 h. Blood samples (400 μL) were collected into heparinized tubes. The samples were centrifuged for 15 min (5 °C, 2000 rpm), and the plasma was separated and transferred into polypropylene tubes. Post-mortem samples of whole intestine, lungs, and kidneys from 4 mice were obtained at 0.5; 1; 4; 8; and 12 h. All the samples were stored at −18 °C, until analyzed by high performance liquid chromatography (HPLC).

The frozen plasma and tissue samples were thawed at room temperature. The freeze−thaw stability was verified at three levels of concentrations (0.08, 2.4, and 14.3 μg/mL for plasma; 0.05, 5.0, and 10.0 μg/mL for kidney, lung, and intestinal tissues) after three freeze−thaw cycles. No tendency of degradation was observed. The samples were processed using the method described by Gonzalez[29] with modifications. Portions of 100 μL of plasma were placed into 5 mL glass tubes, spiked with 20 μL of MAR (5 μg/mL) as internal standard. CIP and MAR were submitted to solid phase extraction on reversed-phase C18 cartridges (StrataTM × 50 μm Polymeric Sorbent, 100 mg/mL; Phenomenex), which were preconditioned with 0.5 mL of methanol and 0.5 mL of water. The samples were applied to the cartridges, washed with 3 mL of Milli-Q water, and eluted with 2 mL of methanol. The eluate was collected and evaporated to dryness at 40 °C under vacuum (Speed-Vac, Savant, USA).

The tissue samples were carefully minced and portions of lungs (50 mg), kidneys (150 mg), and intestine (150 mg) were used and placed into 5 mL glass tubes, spiked with 37.5 μL of DAN (1 μg/mL) and 350 mL of phosphate buffer pH 7 and vortexed during 30 s. The samples were extracted using 2 mL of acetonitrile, stirring for 10 min, and centrifuged (10 min, 5 °C, 3800g). The supernatants were transferred into glass tubes and evaporated to dryness under vacuum at 40 °C. The dried extracts were reconstituted with 200 μL of MeOH and 800 μL phosphate buffer and then were subjected to a solid phase extraction process, similar to that described above.

All dried extracts were reconstituted in variable volumes of mobile phase and then injected into the HPLC system for quantification of CIP.

Bacterial Strain. A strain of *Salmonella enterica* serotype Enteritidis, originally isolated from a patient with a severe bacterial infection and hospitalized at the intensive care unit of the Santamarina Hospital, Tandil, Argentina, was used for challenging the mice.

Minimal Inhibitory Concentration (MIC). MIC was determined for solutions of CIP-complex, CIP (prepared from CIP hydrochloride), and aluminum (prepared from aluminum chloride-6-hydrate). The *Salmonella enterica* serotype Enteritidis MIC testing conditions were based on broth dilution, using Müeller−Hinton broth supplemented with calcium and magnesium employing a final inoculum of 1×10^5 CFU/mL. Samples were incubated over 18 h at 35 °C. The control strain used for the technique validation to obtain the MIC for *Salmonella* was *Escherichia coli* ATCC 25922 according to NCCLS 2002 policy.[30] The MICs were defined as the lowest concentration of the antibiotics that gave no visible growth and were performed in triplicate.

Infection Model. Twenty mice were infected with the intragastric administration of 300 μL of a PBS suspension containing 5×10^7 CFU/mL of *Salmonella* Enteritidis in the logarithmic phase of growth, during 2 days (once daily), to allow the bacteria to be implanted in the gut and to induce sepsis, which led to endotoxic shock. One hundred percent of mice developed septicemia and died within a period of 8−12 days after bacterial inoculation.

Assessment of the Disease Evolution Assay. Fifty mice were infected as described in the previous item and divided into five groups. Forty eight hours postinoculation, each group was intragastrically treated for 5 days, with 300 μL of distilled water (control group), CIP-complex solution once daily, CIP-complex solution twice daily, reference solution once daily, and reference solution twice daily. Every mouse was observed, and the percentage survival was daily recorded for 15 days. Additionally, the clinical score was based on a scale of 0−5,

according to Biswas,[31] as follows: a normal and unremarkable condition was scored as 5; slight illness, defined as lethargy and ruffled fur, was scored as 4; moderate illness, defined as severe lethargy, ruffled fur, and hunched back, was scored as 3; severe illness, with the above signs plus exudative accumulation around partially closed eyes, was scored as 2; a moribund state was scored as 1; and death was scored as 0.

Equipment, Chromatographic Conditions, and Standard Solutions. Stock solutions of CIP (1000 μg/mL) were prepared in methanol. Working solutions (100 μg/mL) of CIP were prepared by diluting several milliliters of stock solution with phosphate buffer.

Stock solutions of internal standard were prepared by dissolving MAR (100 μg/mL) or DAN (100 μg/mL) in phosphate buffer. The stock solutions of MAR and DAN were diluted in phosphate buffer to give working internal standard solutions, which were used to fortify test samples. All standards and working solutions were stored in dark at 0−4 °C. All solutions prepared for HPLC were passed through a 0.45 μm cellulose acetate filter before use. The chromatography conditions reported by Gonzalez were used.[29]

Four seven-point calibration curves were prepared over a range of 0.08−14.3 μg/mL (plasma), 0.025−10 μg/g (kidney), and 0.05−10 μg/g (lung and intestine). The linearity, obtained from squared correlation coefficient, r^2, was 0.9858, 0.9993, 0.9962, and 0.9998 for plasma, lung, kidney, and intestine, respectively. The levels of sensitivity were 80 ng/mL for plasma, 25 ng/g for kidney, and 50 ng/g for lung and intestine. Mean plasma (μg/mL) and tissue (μg/g) CIP concentrations versus post administration time were obtained using Origin 6.0. The maximum plasma (or tissue) concentration (C_{max}), the time at C_{max} plasma (or tissue) (T_{max}), and the AUC_{0-12} were calculated using the software PK Solution 2.0. The statistical analysis of pharmacokinetics and tissue distribution parameters between complex and reference groups was carried out by Student's *t*-test using Graph Pad Instant3.0. The results were expressed as mean ± standard deviation (SD) with the 95% confidence interval and the *p*-value < 0.05.

RESULTS

MIC. MIC obtained for *E. coli* ATCC 25922 was 0.006 mg/L for both, CIP and CIP-complex. In addition, MIC obtained for *S. enterica* serotype Enteritidis of both CIP and CIP-complex resulted in a value of 0.25 mg/L. No antimicrobial activity against any bacteria was evident for the aluminum solution (MIC ≥ 1024 mg/L). These results confirm the bactericidal action of CIP against the pathogen used in the experimental infection as well as the lack of bactericidal activity of aluminum.

Systemic Exposure and Tissue Distribution Studies. Concentrations of CIP in plasma were determined at various intervals after intragastric administration of a 5 mg CIP/kg as a single dose of CIP-complex and reference solutions. Figure 2 shows the mean plasma concentration−time profiles that were obtained. As can be seen there, CIP was rapidly absorbed from both solutions. The C_{max} value for CIP-complex (3.60 μg/mL) was 33% higher than reference (2.60 μg/mL) ($p < 0.014$). In addition, an earlier T_{max} (1.0 h) was observed from CIP-complex as compared to the reference (1.5 h). After C_{max}, a faster decrease was observed in plasma levels of CIP-complex; while a more flattened profile was observed in the reference group. Six hours postadministration, the elimination profiles in both groups became practically equal with levels of 0.7 μg/mL 12 h postadministration. The AUC_{0-12} of the reference (13.2 μg.h/mL) was slightly higher than that of CIP-complex (11.6 μg.h/mL). Basic predictive pharmacokinetic and pharmacodynamic (PK/PD) values, such as C_{max}/MIC and AUC_{0-12}/MIC, which can be used to predict efficacy and the impact on bacterial resistance, were also calculated from plasma concentration data. The PK/PD integration C_{max}/MIC as efficacy index was calculated after both treatments, obtaining a value of 1040 for the reference group (CIP alone) and 1440 (+38%) for the experimental group (CIP-complex). However, similar values 52.8 and 46.4 were obtained after comparison of AUC/MIC in CIP-complex versus CIP-reference groups, respectively.

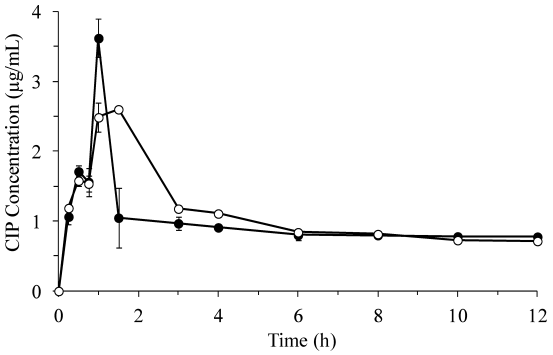

Figure 2. Mean plasma concentration−time profiles of CIP following a single oral administration of complex (—●—) and reference solutions (—○—), in Balb-C mice. The solutions were intragastrically administered as a single dose (equivalent to 5 mg CIP/kg). Data are expressed as mean ± SD for 4 mice. Statistical significance (*) $p < 0.014$.

CIP distribution in intestine, lung, and kidney versus time profiles are shown in Figure 3. The maximum levels in both groups were obtained between 0.5 and 1 h in all the tissues. The maximum concentrations of CIP obtained via CIP-complex were 447% (intestine), 93% (kidney), and 44% (lung) higher than in reference, at the same time.

Assessment of Disease Evolution. Antimicrobial treatments started 48 h after the last bacterial inoculation. Percent survival of mice is shown in Figure 4. The infection model was effective in inducing death, and 100% mortality between 5 and 12 days postinfection was observed in the control group. The parameter of therapeutic efficacy of antimicrobial treatment was the survival of mice assessed for a period of 15 days after treatment. At that time, the survival percentage of the group administered with CIP-complex twice daily (40%) was higher than that of the reference administered twice daily (20%). In addition, the state of health of the surviving mice in this CIP-complex group was higher (5 of 5 score) than that of the reference group (3 of 5 score). However, 30% of survival was observed in the group administered with CIP-complex once daily, compared with 100% mortality in the group administered with the reference solution once daily. Interestingly, survival in the group administered with CIP-complex once daily was even higher than that of the reference administered twice daily.

DISCUSSION

There are several ways to improve antibiotic treatments. In particular, intravenous administration in long-circulating liposomes of CIP results in a considerable enhancement of its therapeutic efficacy compared with CIP in the free form,[32] mainly due to enhanced penetration to infected sites and/or pharmacokinetics changes after liposomal encapsulation. In

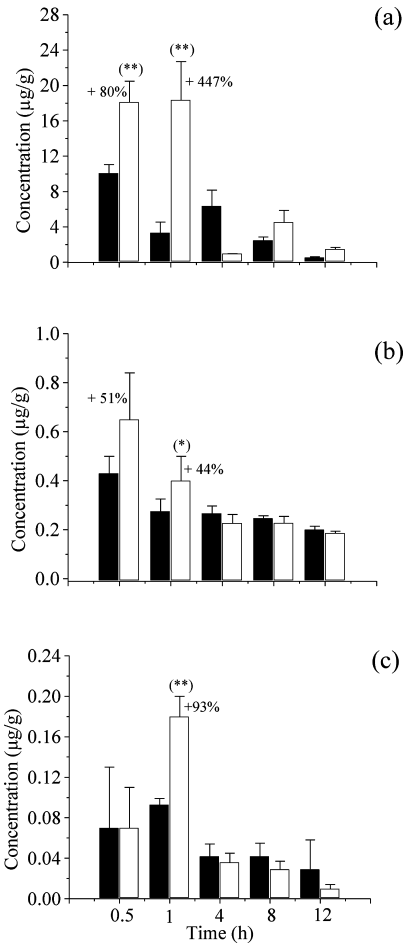

Figure 3. CIP levels in intestine (a), lung (b), and kidney (c) after a single intragastric administration of CIP-complex (white bar) and reference solutions (black bar) in Balb-C mice at a dose of 5 mg CIP/kg. Statistical significance (**) $p < 0.01$ and (*) $p < 0.05$. Error bars represent standard deviations of quadruplicate.

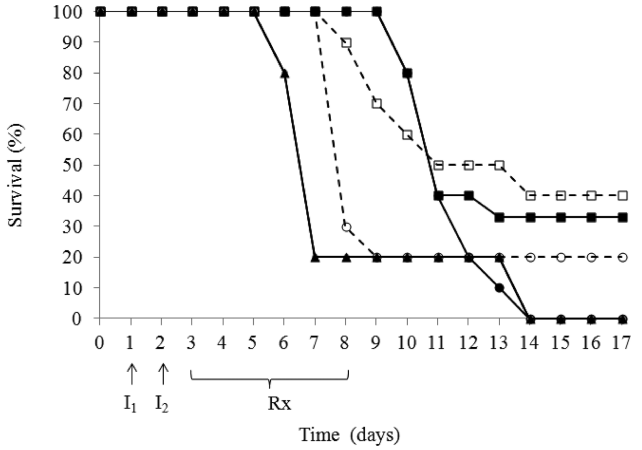

Figure 4. Percent survival of Balb-C mice (n = 10/group) infected during 2 days (I_1 and I_2) with a lethal dose of *Salmonella enteritidis*: group administered with CIP-complex solution once daily (—■—); group administered with CIP-complex solution twice daily (—□—); group administered with reference solution once daily (—●—); group administered with reference solution twice daily (—○—); control group receiving distilled water (—▲—). Rx: duration of antibiotic therapy.

fact, liposomal CIP was more effective than free CIP in the treatment of *Klebsiella pneumoniae* in rats,[33] murine salmonellosis in mice[34] and rats with pneumonia[35] when they are administered as intravenous solutions. Drug delivery systems containing CIP incorporated into PEGylated liposomes or niosomes for treatment of respiratory infections have been recently reported although efficacy was not yet informed.[36,37] Some of these systems were reported almost 20 years ago, but despite their success, no formulations based on liposomes are commercially available today. In addition, these systems are not suitable for oral administration, which is the most frequent and convenient administration route for drugs.

On the contrary, drug delivery systems of CIP for oral administration are commercially available. These systems are as safe and effective as the conventional immediate release systems of CIP with the advantage that they allow once-daily administration while maintaining therapeutic serum levels of CIP. However, at present, no in vivo superiority has been demonstrated either in humans or animal models.[38−41] The use of salts, complexes, and polymorphs is a widely used strategy to optimize the performance of drugs. The selection of this for a drug candidate is recognized as an essential step in the preclinical phase of modern drug development. In fact, CIP is given orally as the hydrochloride or base, by intravenous infusion as the lactate, and in eye drops, eye ointment, or ear drops as the hydrochloride.[14]

In this study, we evaluated the bioavailability of CIP administered as the high solubility CIP-complex in comparison with CIP hydrochloride salt used as reference.

The stability of a CIP-complex solution was previously evaluated by determining the MIC against *Staphylococcus aureus* and *Escherichia coli* for a period of 10.5 months.[21] The results indicated that, over the assayed period, CIP-complex solutions were as stable as equimolar solutions of CIP hydrochloride since their antimicrobial activity remains the same and unchanged.

Our results showed that the increase in solubility did impact absorption patterns of CIP. Since solubility often limits the rate of absorption of a compound, one expects that improving the solubility of a poorly soluble compound would shift T_{max} to the left. This was the case for the CIP-complex, which not only reached the systemic circulation more quickly than the reference but also at significantly higher C_{max}. Besides, the mean CIP plasma concentration in the CIP-complex was not negatively affected by the presence of aluminum in stoichiometric amounts of CIP/aluminum, 3:1. There are reports regarding a reduction in oral bioavailability of CIP when a dose is given concomitantly with metallic antacid containing aluminum and/or magnesium.[14] However, a high molar excess of metal with respect to CIP is present in a typical dose of antacid. In particular, the strong adsorption of quinolones by aluminum hydroxide precipitated in the small intestine has been suggested as the factor contributing to the reduction in fluoroquinolones bioavailability.[42,43]

The poor solubility of CIP at intestinal pH range plays an important role in the reduced oral whole bioavailability of CIP.[2] Harder et al.[44] reported that the AUC obtained after releasing CIP in the stomach decreased to 37%, 23%, and 7% when it is released in jejunum, ileum, and colon. This can be explained by the pH-dependent in vivo dissolution of CIP. In fact, CIP is soluble in the stomach, either administered as the hydrochloride or as the free base. However, as it reaches the absorption site (duodenum and jejunum) its low solubility

drives to precipitation. This behavior would occur even for the lowest dose of CIP (250 mg, as hydrochloride), and as the dose increases, a higher precipitated fraction is expected. This problem was overcome by CIP-complex since it allows the complete dissolution at intestinal pHs for doses of 250 and 500 mg of CIP.[3] Previous results showed that intestinal permeability of CIP from CIP-complex did not show significant differences when compared with CIP reference solutions.[45] Besides, preliminary studies in bicompartimental chambers showed the same trend. Then, the decrease in T_{max} would be in agreement with an increased offer of CIP in the absorption site when it is given as CIP-complex and could be explained by its high solubility and not assigned to intestinal permeability differences.[3]

The sudden decrease of plasmatic CIP concentrations observed after C_{max} can be ascribed to a fast distribution, which, in fact, conducted to higher tissue levels. In contrast, plasmatic concentrations of the reference showed a flattened profile that can be assigned to the slow dissolution rate of CIP precipitated at intestinal pHs. This behavior was previously observed in humans by Tartaglione after the oral administration of CIP doses from 250 to 1000 mg.[7] They observed that the events leading up to the absorption step were rate limited after sequentially increasing oral doses of CIP, with the absorption phase fitted by a zero-order equation. The gastrointestinal absorption data for other antibiotics, including sulfisoxazole, griseofulvin, and erythromycin, have been described by a zero-order process and were likely due to dissolution problems.[46−48]

The determination of survivorship in mouse experimental septicemia is a primary model for the evaluation by efficacy of antimicrobial agents.[49] In our septicemia model, the *Salmonella* Enteritidis is mainly installed into intestine and then moves to other tissues (hematopoietic, lung, kidney, etc.) releasing endotoxins, which generates a septic shock with consequent death. Scientific literature suggests that the therapeutic activities of fluoroquinolones in experimental infections are related to their pharmacokinetic behavior.[50,51] Bactericidal activity of CIP for Gram negative bacteria such as *Salmonella* is concentration-dependent.[52] Thus, the higher concentrations of CIP detected in the intestine after treatment with CIP-complex could be responsible for the higher efficacy observed in the groups administered with CIP-complex once and twice daily when compared with their respective reference groups.

The superiority of CIP-complex is more striking when the groups administered with CIP-complex once daily and reference twice daily are compared since 5 mg CIP/kg/day allows a 30% survival with CIP-complex while 10 mg CIP/kg/day allows only 20% survival with the reference. Besides, the reference solution failed to avoid death (0% survival) when it was administered once daily.

The higher efficacy of CIP-complex could be linked to the differences observed in quantities of CIP found in intestine at 1 and 2 h since this organ is the principal infection site of *Salmonella*.

This study also suggests that serum, kidney, lung, and intestine levels of CIP following oral administration of CIP-complex achieve levels that should inhibit systemic infections caused by several strains since levels of CIP were higher or equivalent. As usual, for pharmaceutical derivatives such as salts and complexes, in our study, the plasma and tissue samples were analyzed only for the active moiety CIP regardless it if was free or complexed with aluminum. This is because a dynamic equilibrium between complexed and uncomplexed forms of CIP is expected in solution in the preabsorptive events, being that free CIP species are the most favored for intestinal absorption. However, given the high affinity between CIP and aluminum, some absorption of complexed species is probable and future studies should investigate this aspect.

Results reported here, where CIP was compared to CIP-complex, showed that their MICs are equivalent against *E. coli* (0.006 mg/L) and *S.* Enteritidis (0.25 mg/L). In the MIC studies, we also consider aluminum alone. This is important because equal MIC in CIP and CIP-complex may be due to the reduced activity of CIP after complexation and the compensation for the loss by the complex material. Notice that no bactericidal activity was observed for aluminum against *E. coli* or *S.* Enteritidis. Similar results were reported by Ma et al.[53] who described that aluminum alone, up to concentrations of 2 mM did not produce any measurable bactericidal effect against *E. coli*. An inverse relationship between the antibacterial effect of CIP and the presence of an excess of aluminum was also described. In fact, a molar excess of 4300-fold of aluminum with respect to CIP yielded a 50% reduction in the bactericidal rate; however, no reduction was observed with a molar excess of 100 times. Then, the in vivo better performance showed for the oral administration of CIP-complex when compared with CIP is not predictable from microbiological tests and would be most probably related to the solubility improvement.[20]

CONCLUSIONS

Pharmacokinetic and tissue distribution studies showed that the plasma and tissue CIP concentrations at 0.5 and 1 h are significantly higher following CIP-complex administration. In addition, a positive tendency toward greater effectiveness of CIP can be viewed from CIP-complex solution compared to reference in the *Salmonella* infection model that was used and that was given both once and twice daily. In both cases, a reduction in the number of deaths was observed as well as a better health state at the end of the experiment.

Although AUC values were slightly lower in CIP-complex, the efficacy results could be linked to differences in greater plasmatic and tissue concentrations achieved with CIP-complex. The possible clinical relevance with respect to effectiveness of CIP from CIP-complex solutions in gastrointestinal pathologies could be considered since CIP is an antibiotic with concentration-dependent bactericidal activity for Gram negative bacteria.[52] However, a detailed study using an appropriate regime is necessary to assess the microbiological cure.

To the best of our knowledge, this is the first report in which a pharmaceutical derivative of CIP proves to be more effective than CIP after oral administration. This is interesting since CIP-complex has several advantages, for example, it can be formulated as more dose-efficient immediate release tablets with fast, complete, and pH-independent dissolution rate. Stability studies will be further required to establish shelf life for oral solid or liquid formulations.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Word Health Organization. 17th WHO Model List of Essential Medicines. http://www.who.int/medicines/publications/essentialmedicines/en/index.html (accessed April 2012).

(2) Olivera, M. E.; Manzo, R. H.; Junginger, H.; Midha, K.; Shah, V.; Stavchansky, S.; Dressman, J.; Barends, D. Biowaiver monographs for immediate release solid oral dosage forms: ciprofloxacin hydrochloride. *J. Pharm. Sci.* **2011**, *100*, 23−33.

(3) Breda, S. A.; Jimenez Kairuz, A. F.; Manzo, R. H.; Olivera, M. E. Solubility behavior and biopharmaceutical classification of novel high-solubility ciprofloxacin and norfloxacin pharmaceutical derivatives. *Int. J. Pharm.* **2009**, *371*, 106−113.

(4) Drusano, G. L.; Standiford, H. C.; Plaisance, K.; Forrest, A.; Leslie, J.; Caldwell, J. Absolute oral bioavailability of ciprofloxacin. *Antimicrob. Agents Chemother.* **1986**, *30*, 444−446.

(5) Brunner, M.; Sta, H.; Moller, J. G.; Schrolnberger, C.; Erovic, B.; Hollenstein, U.; Zeitlinger, M.; Eichler, H. G.; Muller, M. Target site concentrations of ciprofloxacin after single intravenous and oral doses. *Antimicrob. Agents Chemother.* **2002**, *46*, 3724−3730.

(6) Hoffken, G.; Lode, H.; Prinzing, C.; Borner, K.; Koeppe, P. Pharmacokinetics of ciprofloxacin after oral and parenteral administration. *Antimicrob. Agents Chemother.* **1985**, *27*, 375−379.

(7) Tartaglione, T. A.; Raffalovich, A. C.; Poynor, W. J.; Espinel-Ingroff, A.; Kerkering, T. M. Pharmacokinetics and tolerance of ciprofloxacin after sequential increasing oral doses. *Antimicrob. Agents Chemother.* **1986**, *29*, 62−66.

(8) Plaisance, K. I.; Drusano, G. L.; Forrest, A.; Bustamante, C.; Standiford, H. C. Effect of dose size on bioavailability of ciprofloxacin. *Antimicrob. Agents Chemother.* **1987**, *31*, 956−958.

(9) USP 33-NF 28. *The United States Pharmacopeia: The National Formulary*; The United States Pharmacopeial Convention, Inc.: Rockville, MD, 2010.

(10) Firestone, B. A.; Dickason, M. A.; Tran, T. Solubility characteristics of three fluoroquinolone ophthalmic solutions in an in vitro tear model. *Int. J. Pharm.* **1998**, *164* (1), 119−128.

(11) Eiferman, R. A.; Snyder, J. P.; Nordquist, R. E. Ciprofloxacin microprecipitates and macroprecipitates in the human corneal epithelium. *J. Cataract. Refract. Surg.* **2001**, *27* (10), 1701−1702.

(12) De Benedetti, G.; Brancaccio, A. Corneal deposit of ciprofloxacin after laser assisted subepithelial keratomileusis procedure: a case report. *J. Ophthalmol.* **2010**, *2010*, 296034.

(13) Madhavan, H. N.; Rao, S. K.; Joseph, P. R.; Sulochana, K. N. Antibacterial activity of the white precipitate formed on the corneal surface after treatment with ciprofloxacin ophthalmic solution. *Cornea* **1999**, *18* (5), 549−552.

(14) Sweetman, S. C. Martindale. *The Complete Drug Reference*, 36th ed.; Pharmaceutical Press: London, U.K., 2009.

(15) Trissel, L. A. *Handbook of Injectable Drug*, 9th ed.; Amercian Society of Hospital Pharmacists: Bethesda, MD, 1996.

(16) Sarma, B.; Chen, J.; His, H. Y.; Myerson, A. S. Solid forms of pharmaceuticals: Polymorphs, salts and cocrystals. *Korean J. Chem. Eng.* **2011**, *28*, 315−322.

(17) Olivera, M. E.; Mazzieri, M. R.; Manzo, R. H. New pharmaceutical derivatives of fluoroquinolones. Hydrochlorides of aluminium complexes of ciprofloxacin and norfloxacin. *STP Pharma Sci.* **2000**, *10*, 251−256.

(18) Manzo, R. H.; Mazzieri, M. R.; Olivera, M. E. Preparación de complejos en estado sólido de clorhidratos de antimicrobianos fluoroquiolónicos (AMFQs) con aluminio. Argentinian Patent, AR007762B1, 2005.

(19) Chattah, A. K.; GarroLinck, Y.; Monti, G.; Levstein, P.; Breda, S. A.; Manzo, R. H.; Olivera, M. E. NMR and IR characterization of the aluminium complexes of norfloxacin and ciprofloxacin fluoroquinolones. *Magn. Reson. Chem.* **2007**, *45*, 850−859.

(20) Alovero, F. L.; Olivera, M. E.; Manzo, R. H. In vitro pharmacodynamic properties of a fluoroquinolone pharmaceutical derivative: hydrochloride of ciprofloxacin−aluminium complex. *Int. J. Antimicrob. Agents* **2003**, *21*, 446−451.

(21) Allemandi, D. A.; Alovero, F. L.; Manzo, R. H. Formulation of a neutral solution of ciprofloxacin upon complexation with aluminum. *Farmaco* **1999**, *54*, 758−760.

(22) Olivera, M. E.; Bistoni, A.; Anun, H.; Salinas, A.; Manzo, R. H. Ototopic ciprofloxacin in a glycerin vehicle for the treatment of acute external otitis. *Ear, Nose Throat J.* **2004**, *83*, 625−630, 638.

(23) Veldman, K.; Cavaco, L. M.; Mevius, D.; Veldman, K.; Cavaco, L. M.; Mevius, D.; Battisti, A.; Franco, A.; Botteldoorn, N.; Bruneau, M.; Perrin-Guyomard, A.; Cerny, T.; De Frutos Escobar, C.; Guerra, B.; Schroeter, A.; Gutierrez, M.; Hopkins, K.; Myllyniemi, A. L.; Sunde, M.; Wasyl, D.; Aarestrup, F. M.. International collaborative study on the occurrence of plasmid-mediated quinolone resistance in *Salmonella enterica* and *Escherichia coli* isolated from animals, humans, food and the environment in 13 European countries. *J. Antimicrob. Chemother.* **2011**, *66* (6), 1278−1286.

(24) Johnson, L. R.; Gould, L. H.; Dunn, J. R.; Berkelman, R.; Mahon, B. E. Foodnet travel working group, Salmonella infections associated with international travel: a foodborne diseases active surveillance network (FoodNet) study. *Foodborne Pathog. Dis.* **2011**, *8* (9), 1031−1037.

(25) Kim, S. *Salmonella serovars* from foodborne and waterborne diseases in Korea, 1998−2007: total isolates decreasing versus rare serovars emerging. *J. Korean Med. Sci.* **2010**, *25* (12), 1693−1699.

(26) Hendriksen, R. S.; Vieira, A. R.; Karlsmose, S.; Lo Fo Wong, D. M.; Jensen, A. B.; Wegener, H. C.; Aarestrup, F. M. Global monitoring of *Salmonella serovar* distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. *Foodborne Pathog. Dis.* **2011**, *8* (8), 887−900.

(27) Guide to the care and use of experimental animals (Canadian Council on Animal Care), 1993.

(28) Institutional Animal Care and Use Committee Guidebook (ARENA/OLAW IACUC Guidebook, National Institutes of Health, 2002.

(29) Gonzalez, C.; Moreno, L.; Small, J.; Douglas, G.; Sanchez Bruni, S. F. A liquid chromatographic method, with fluorometric detection, for the determination of enrofloxacin and ciprofloxacin in plasma and endometrial tissue of mares. *Anal. Chim. Acta* **2006**, *569*, 227−234.

(30) National Committee for Clinical Laboratory Standards Performance Standards for Antimicrobial Susceptibility Testing; MIC Testing, Twelfth Informational Supplement (NCCLS); M100-S12; 22 (1), 2002.

(31) Biswas, B.; Adhya, S.; Washart, P.; Trostel, A. N.; Powell, B.; Carlton, R.; Merril, C. R. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. *Infect. Immun.* **2002**, *70* (1), 204−210.

(32) Bakker-Woudenberg, I. A.; Schiffelers, R. M.; Storm, G.; Becker, M. J.; Guo, L. Long-circulating sterically stabilized liposomes in the treatment of infections. *Methods Enzymol.* **2005**, *391*, 228−260.

(33) Bakker-Woudenberg, I. A.; Ten Kate, M. T.; Guo, L.; Working, P.; Mouton, J. W. Improved efficacy of ciprofloxacin administered in polyethylene glycol-coated liposomes for treatment of Klebsiella pneumoniae pneumonia in rats. *Antimicrob. Agents Chemother.* **2001**, *45* (5), 1487−1492.

(34) Magallanes, M.; Dijkstra, J.; Fierer, J. Liposome-incorporated ciprofloxacin in treatment of murine salmonellosis. *Antimicrob. Agents Chemother.* **1993**, *37* (11), 2293−2297.
(35) Chono, S.; Tanino, T.; Seki, T.; Motrimoto, K. Efficient drug delivery to alveolar macrophages and lung epithelial lining fluid following pulmonary administration of liposomal ciprofloxacin in rats with pneumonia and estimation of its antibacterial effects. *Drug Dev. Ind. Pharm.* **2008**, *34* (10), 1090−1096.
(36) Chono, S.; Suzuki, H.; Togami, K.; Motrimoto, K. Efficient drug delivery to lung epithelial lining fluid by aerosolization of ciprofloxacin incorporated into PEGylated liposomes for treatment of respiratory infections. *Drug Dev. Ind. Pharm.* **2011**, *37* (4), 367−372.
(37) Moazeni, E.; Gilani, K.; Sotoudegan, F.; Pardakhty, A.; Najafabadi, A. R.; Ghalandari, R.; Fazeli, M. R.; Jamalifar, H. Formulation and in vitro evaluation of ciprofloxacin containing niosomes for pulmonary delivery. *J. Microencapsul.* **2010**, *27* (7), 618−627.
(38) Rashid, M.; Weintraub, A.; Nord, C. E. Comparative effects of the immediate and the extended release formulations of ciprofloxacin on normal human intestinal microflora. *J. Chemother.* **2011**, *23* (3), 145−149.
(39) Mirone, V.; Fusco, F.; Taglialatela, D.; Verze, P.; Di Vito, C.; Lotti, T.; Imbimbo, C.; Emeron, A. Efficacy and safety of ciprofloxacin XR 1000 mg once daily versus ciprofloxacin 500 mg twice daily in the treatment of complicated urinary tract infections. *J. Chemother.* **2009**, *21* (6), 651−660.
(40) Waugh, J.; Keating, G. M. Ciprofloxacin extended release: in the treatment of urinary tract infections and uncomplicated pyelonephritis. *Drugs Aging* **2004**, *21* (1), 55−64.
(41) Blondeau, J. M. Current issues in the management of urinary tract infections: extended-release ciprofloxacin as a novel treatment option. *Drugs* **2004**, *64* (6), 611−628.
(42) Tanaka, M.; Kurata, T.; Fujisawa, C.; Ohshima, Y.; Aoki, H.; Okazaki, O.; Hakusui, H. Mechanistic study of inhibition of levofloxacin absorption by aluminum hydroxide. *Antimicrob. Agents Chem.* **1993**, *37* (10), 2173−2178.
(43) Sultana, N.; Arayne, S. M.; Hussain, F. In vitro monitoring of ciprofloxacin antacids interactions by UV & HPLC. *Pak. J. Pharm. Sci.* **2005**, *18* (4), 23−31.
(44) Harder, S.; Fuhr, U.; Beermann, D.; Staib, H. Ciprofloxacin absorption in different regions of the human gastrointestinal tract. Investigations with the HF-capsule. *Br. J. Clin. Pharmacol.* **1990**, *30*, 35−39.
(45) Olivera, M. E.; Allemandi, D. A.; Manzo, R. H. Velocidad de disolución intrínseca y permeabilidad intestinal de complejos metálicos de norfloxacino y ciprofloxacino en relación a la formulación de comprimidos. *Acta Farm. Bon.* **2000**, *19* (3), 185−192.
(46) Bates, T. R.; Carrigan, P. J. Apparent absorption kinetics of micronized griseofulvin after its oral administration on single- and multiple-dose regimens to rats as a corn oil-in-water emulsion and aqueous suspension. *J. Pharm. Sci.* **1975**, *64* (9), 1475−1481.
(47) Colburn, W. A.; Di Santo, A. R.; Gibaldi, M. Pharmacokinetics of erythromycin on repetitive dosing. *J. Clin. Pharmacol.* **1977**, *17*, 592−600.
(48) Kaplan, S. A.; Weinfeld, R. E.; Abruzzo, C. W; Lewis, M. Pharmacokinetic profile of sulfisoxazole following intravenous, intramuscular, and oral administration to man. *J. Pharm. Sci.* **1972**, *61* (5), 773−778.
(49) Zal, O.; O'Reilly, T. Animal models in the evaluation of antimicrobial agents. *Antimicrob. Agents Chemother.* **1991**, *35*, 1527−1531.
(50) Fu, K.; Lafredo, S.; Foleno, B.; Isaacson, D. M.; Barrett, J. F.; Tobia, A. J.; Rosenthale, M. E. In vitro and in vivo antibacterial activities of levofloxacin (L-ofloxacin), an optically active ofloxacin. *Antimicrob. Agents Chemother.* **1992**, *36*, 860−866.
(51) Craig, W. Pharmacokinetic:pharmacodynamic parameters: Rationale for antibacterial dosing of mice and men. *Clin. Infect. Dis.* **1998**, *26*, 1−12.
(52) Martinez, M.; McDermott, W. Pharmacology of the fluorquinolones: a perspective in domestic animals. *Vet. J.* **2006**, *172*, 10−28.
(53) Ma, H. H. M.; Chiu, F. C. K.; Li, R. C. Mechanistic investigation of the reduction in antimicrobial activity of ciprofloxacin by metal cations. *Pharm. Res.* **1997**, *14* (3), 366−370.