Ciprofloxacin: a novel therapeutic agent for iron overload?

Siprofloksasin: demir yüklenmesi için yeni bir terapötik ajan mı?

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Abstract

Objective: Major thalassemia is one of the hematological diseases requiring multiple blood transfusions, which results in iron overload in the liver, heart and other organs. Current iron chelation therapy consists of intravenous (IV) deferoxamine and oral deferasirox and deferiprone. Although these chelators are effective, many side effects are reported. In the present study, the iron-chelating effect of ciprofloxacin with good oral absorption was investigated.

Material and Methods: Thirty male albino Wistar rats were used for the study. Ciprofloxacin (7 or 14 mg/kg per day) was administered simultaneously with iron (0.03 g/kg per day) or after one-month administration of iron. Ciprofloxacin effect on iron absorption in the liver and heart was studied carefully using atomic absorption.

Results: A significant decrease in the liver and heart iron following the ciprofloxacin (14 mg/kg per day) administration was observed, when compared with the control group. This ciprofloxacin-induced tissue iron depletion was more pronounced when it was administered simultaneously with iron, when it was administered for a longer duration (2 months rather than 1 month) and when it was given in higher doses (14 mg/kg per day).

Conclusion: Administration of ciprofloxacin may help to decrease the burden of parenteral administration, thereby improving compliance and also the life expectancy of thalassemic patients. *(Turk J Hematol 2009; 26: 114-7)*

Key words: Thalassemia, iron overload, ciprofloxacin, atomic absorption, chelator

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Özet

Amaç: Talasemi majör, karaciğer, kalp ve diğer organlarda demir yüklenmesiyle sonuçlanan, çoklu kan transfüzyonları gerektiren hematolojik hastalıklardan biridir. Güncel demir şelasyon tedavisi, intravenöz (IV) defroksamin ve oral deferasiroks ve deferipron uygulamasını içermektedir. Bu şelatörlerin etkili olmalarına karşın, birçok yan etkileri bulunmaktadır. Bu çalışmada, iyi oral absorbsiyonlu siprofloksasinin demir bağlayıcı etkileri araştırılmıştır.

Yöntem ve Gereçler: Bu çalışmada 30 erkek Wistar sıçanı kullanıldı. Siprofloksasin (7 veya 14 mg/kg/gün), demirle aynı zamanda (0.03 g/kg/gün) veya 1 aylık demir uygulaması sonrası uygulanmıştır. Siprofloksasinin karaciğer ve kalpteki demir absorbsiyonu üzerine olan etkisi atomik absorbsiyon kullanılarak dikkatlice çalışılmıştır.

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Bulgular: Kontrol grubuyla karşılaştırıldığında, siprofloksasin uygulamasını (14 mg/kg/gün) takiben karaciğer ve kalp demirinde anlamlı bir düşüş gözlenmiştir. Siprofloksasinle indüklenen bu doku demir deplesyonu yüksek dozda daha belirgindir.
Sonuç: Siprofloksasin uygulaması, uyumluluğu ve ayrıca talasemik hastaların yaşam beklentisini artırarak, parenteral uygulama yükünü azaltmaya yardımcı olabilir. *(Turk J Hematol 2009; 26: 114-7)*
Anahtar kelimeler: Talasemi, demir yüklenmesi, siprofloksasin, atomik absorbsiyon, şelatör

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Introduction

Patients with thalassemia suffer from iron overload and other complications due to the chronic red blood cell transfusions. This complication results in low life expectancy if they do not receive proper chelating agents such as deferoxamine, which is known as the gold standard for depleting the iron from tissues. Deferoxamine is highly expensive and poorly absorbed from the gastrointestinal tract, and these disadvantages limit its regular use in the clinic. A safe and effective oral iron chelator has been the goal of therapeutic research about this disease for several decades [1]. Deferasirox and deferiprone are two orally-active iron chelators that have been approved for clinical use, but they are not yet easily accessible to all patients [2].

Ciprofloxacin, 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline-carboxylic acid (Figure 1) can form a stable green complex with Fe^{3+} and red complex with Fe^{2+} [3-5]. This complex can lead to several interactions between iron and the absorption of ciprofloxacin [6]. It is believed that formation of ferric ion complexes is the likely cause of the reduction in ciprofloxacin bioavailability in the presence of iron [7].

Thus far, there have not been any clear documents based on investigation of the iron-lowering effect of ciprofloxacin in tissues such as the liver or heart. This led us to study the preventive effect of ciprofloxacin on iron overload in rats, as laboratory animal models of iron overload [8].

Materials and Methods

Animals

Thirty male albino Wistar rats (250-300g) were used for the present study. The animals were purchased from Pasteur Institute of Iran and housed in stainless steel cages, handled daily, and provided with food and water. A 12h light/12h dark cycle was maintained, and the animals were tested during the light cycle. The animal experiments were carried out in accordance with the recommendations from the Declaration of Helsinki and internationally accepted principles in the use of experimental animals. The animals were divided into five groups (A-E), six animals in each.

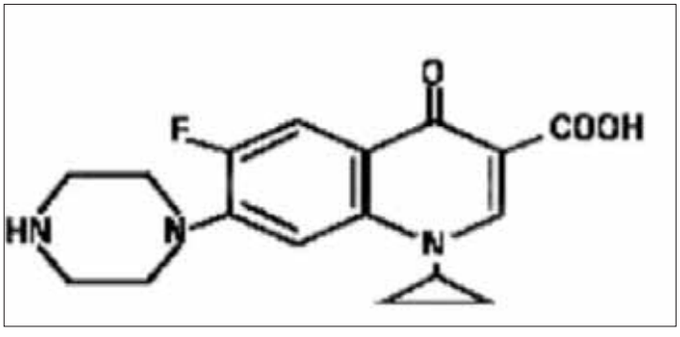

Figure 1. The chemical structure of ciprofloxacin

Drugs and Solvents

Ciprofloxacin was purchased from Biopharmacy Company (Tehran, Iran) and iron dextran (Venofer) from Vinfor (International) Inc. (St. Gallen, Switzerland). Ciprofloxacin was dissolved easily in saline 0.9%.

Experimental Procedure

Thirty male albino Wistar rats were included in this study. They were divided into five groups as follows:

Group A: Control, receiving iron (0.03 g/kg per day iron dextran, intra-peritoneal (i.p.), every 2 days 1 injection) for one month.

Group B: Iron treatment (0.03 g/kg per day, every 2 days 1 injection) for one month and ciprofloxacin (7 mg/kg per day, i.p.) simultaneously for one month.

Group C: Iron treatment (0.03 g/kg per day, every 2 days 1 injection) for one month, followed by ciprofloxacin administration (7 mg/kg per day, i.p.) for one month (ciprofloxacin was started after iron discontinuation).

Group D: Iron treatment (0.03 g/kg per day, every 2 days 1 injection) for one month, followed by ciprofloxacin administration (7 mg/kg per day, i.p.) for two months (ciprofloxacin was started after iron discontinuation).

Group E: Iron treatment (0.03 g/kg per day, every 2 days 1 injection) for one month, followed by ciprofloxacin administration (14 mg/kg per day, i.p.) for one month (ciprofloxacin was started after iron discontinuation).

After the above-mentioned procedures, the rats were sacrificed with ether. Following sacrifice, one lobe of the liver and a portion of the myocardium were perfused with saline to deplete the blood. The removed liver and heart were stored at -20°C until further experiments were performed. For sample preparation, the tissue was homogenized and prepared for atomic absorption using nitric acid digestion. The tissues were dried at 120°C for 24 h. Nitric acid (1.0 ml HNO_3 (1N) per 0.2 g dried tissue) was added and the samples were incubated at 50°C for 16 h and then diluted with deionized water (3.5 ml deionized water per 1.0 ml HNO_3 (1N)). After centrifugation at 2700×g for 10 min, 1 ml aliquots was stored in a sealed con-

tainer at 4°C. Iron determinations were performed using an atomic absorption spectrometer (Varian Spectra 220 Atomic Absorption Spectrometer) [9]. Atomic absorption parameters were set as follows: wavelength: 248.3, slit width: 0.2 nm, gain: 63%, lamp current: 7.0 mA, measurement time: 10.0 sec, flame type: air/acetylene, air flow: 10.00 L/min, acetylene flow: 1.50 L/min, expansion factor: 1.0, and smoothing: 7 points.

Statistical Analysis

The amounts of iron were shown in mg/L and they were depicted as mean ± standard error. For comparing the groups, one-way ANOVA and Tukey Post Hoct tests was used and differences with p values < 0.05 were considered significant.

Results

The quantitation of iron content in the heart and liver of the examined rats showed a significant decrease in iron load in both organs of animals receiving ciprofloxacin, when compared with tissues form rats not receiving ciprofloxacin (Figures 2, 3). This effect of ciprofloxacin was more pronounced when it was administered simultaneously with iron, when it was administered for a longer duration (2 months rather than 1 month) and when it was given in higher doses (14 mg/kg per day), as shown in Table 1.

Discussion

Iron overload in patients with thalassemia and in other patients requiring red blood cell transfusion is an important factor in decreasing life expectancy. There are some evidences that ciprofloxacin may reduce iron load in the body. The mechanism of iron-ciprofloxacin complex formation is related to the reaction between 4-keto and 3-carboxyl groups of ciprofloxacin and iron. At pH 6.0, a 3:1 (ciprofloxacin: Fe^{3+}) complex is formed rapidly. Bidentate chelators typically form 3:1 complexes with the normally six coordinate ferric ion. Thus, the chemistry of the ciprofloxacin-iron interaction is consistent with the following overall reaction [5,6]:

Fe^{2+} + 3 ciprofloxacin +1/4 O_2 + H^+ → Fe^{3+} $(ciprofloxacin)_3$ $+^1/_2$ H_2O

In this study, we used ciprofloxacin as a second generation of fluoroquinolones for this purpose. We found that concurrent injection of ciprofloxacin and iron decreased the iron level in the liver and heart. Treatment with ciprofloxacin (7 mg/kg per day) for two months after discontinuation of iron was almost similar to simultaneous administration of iron and ciprofloxacin (7 mg/kg per day) injection for one month. It should be mentioned that during the treatment with ciprofloxacin (14 mg/kg per day), we observed an apparent darkness of the rats' urine in comparison with control rats, which may implicate the excretion of ciprofloxacin-iron complex from the urine. This may be one explanation for the effectiveness of ciprofloxacin in decreasing iron overload.

Ciprofloxacin is a broad-spectrum antibiotic that is active against both gram-positive and gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division. In the adult population, ciprofloxacin is limited to the treatment of proven serious and life-threatening bacterial infections, such as urinary tract infections, lower respiratory tract infections, acute sinusitis, skin infections, bone and joint infections, infectious diarrhea, and typhoid fever. The determination of dosage for any particular patient must take into consideration the severity and nature of the infection, the susceptibility of the causative organism, the integrity of the patient's host-defense mechanisms, and the

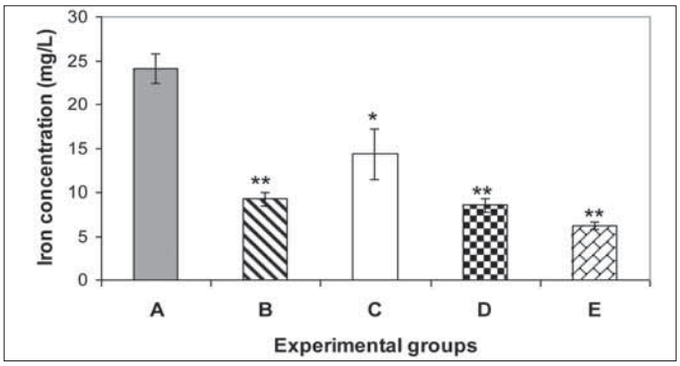

Figure 2. Comparing the iron level of the liver in the investigated groups: A-control group (0.03 g/kg per day iron dextran, i.p., every other day for one month); B- simultaneous i.p. injection of 0.03 g/kg per day iron dextran and 7 mg/kg per day ciprofloxacin every other day for one month; C- One-month i.p. injection of iron dextran (0.03 g/kg per day, every other day), followed by ciprofloxacin administration (7 mg/kg per day, i.p.) for one month; D- One-month i.p. injection of iron dextran (0.03 g/kg per day, every other day), followed by ciprofloxacin administration (7 mg/kg per day, i.p.) for two months; E- One-month i.p. injection of iron dextran (0.03 g/kg per day, every other day), followed by ciprofloxacin administration (14 mg/kg per day, i.p.) for one month.*Significant difference compared with control group (p value < 0.05). **Significant difference compared with control group (p value < 0.001)

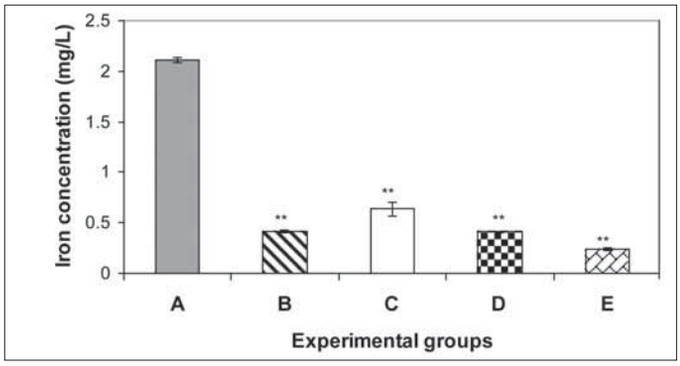

Figure 3. Comparison of the iron level of the heart in the investigated groups: A- control group (0.03 g/kg per day iron dextran, i.p., every other day for one month); B- simultaneous i.p. injection of 0.03 g/kg per day iron dextran and 7 mg/kg per day ciprofloxacin every other day for one month; C- One-month i.p. injection of iron dextran (0.03 g/kg per day, every other day), followed by ciprofloxacin administration (7 mg/kg per day, i.p.) for one month; D- One-month i.p. injection of iron dextran (0.03 g/kg per day, every other day), followed by ciprofloxacin administration (7 mg/kg per day, i.p.) for two months; E- One-month i.p. injection of iron dextran (0.03 g/kg per day, every other day), followed by ciprofloxacin administration (14 mg/kg per day, i.p.) for one month. **Significant difference compared with control group (p value < 0.001)

Table 1. A comparison of the iron levels in the liver and heart between the various groups based on p-values

Organs	P values for the groups					
	B and C	B and D	B and E	C and D	C and E	D and E
Liver	0.0001	0.949	0.0.019	0.0001	0.0001	0.093
Heart	0.001	0.999	0.014	0.001	0.0001	0.024

A- Control group (0.03 g/kg per day iron dextran, i.p., every other day for one month); B- simultaneous i.p. injection of 0.03 g/kg per day iron dextran and 7 mg/kg per day ciprofloxacin every other day for one month; C- One-month i.p. injection of iron dextran (0.03 g/kg per day, every other day), followed by ciprofloxacin administration (7 mg/kg per day, i.p.) for one month; D- One-month i.p. injection of iron dextran (0.03 g/kg per day, every other day), followed by ciprofloxacin administration (7 mg/kg per day, i.p.) for two months; E- One-month i.p. injection of iron dextran (0.03 g/kg per day, every other day), followed by ciprofloxacin administration (14 mg/kg per day, i.p.) for one month

status of renal and hepatic function. The duration of treatment depends upon the severity of infection. The usual duration is 7 to 14 days; however, for severe and complicated infections, more prolonged therapy may be required. A maximum dose of 15 mg/kg (maximum 500 mg per dose) has been administrated for 60 days to treat anthrax [10].

Most of the adverse events reported were described as only mild or moderate in severity, abated soon after the drug was discontinued, and required no treatment. Ciprofloxacin was discontinued because of an adverse event in 1.0% of orally treated patients. The most frequently reported drug-related events, from clinical trials of all formulations, all dosages, all drug-therapy durations, and for all indications of ciprofloxacin therapy were nausea (2.5%), diarrhea (1.6%), abnormal liver function tests (1.3%), vomiting (1.0%), and rash (1.0%).

An Independent Pediatric Safety Committee (IPSC) reviewed all cases of musculoskeletal adverse events. The Food and Drug Administration (FDA) is notifying the makers of fluoroquinolone antimicrobial drugs for systemic use of the need to add a boxed warning to the prescribing information about the increased risk of developing tendinitis and tendon rupture in patients taking fluoroquinolones [11].

With further investigations and confirmation of ciprofloxacin effectiveness in iron overload treatment in human studies and after considering the possible long-term adverse effects, it may be used orally as an effective and preventive drug in iron overloading induced cardiac-liver diseases such as thalassemia or hemochromatosis.

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References

1. Nisbet-Brown E, Olivieri NF, Giardina PJ, Grady RW, Neufeld EJ, Se'chau R, Krebs- Brown AJ, Anderson JR, Alberti D, Sizer KC, Nathan DG. Effectiveness and safety of ICL670 in iron-loaded patients with thalassaemia: a randomized, double-blind, placebo-controlled, dose-escalation trial. Lancet 2003;361:1597-602.
2. Dubey AP, Sudha S, Parakh A. Deferasirox: the new oral iron chelator. Indian Pediatr. 2007;44:603-7.
3. Issopoulos PB. Spectrophotometric determination of trace amounts of iron (III) with norfloxacin as complexing reagent. Analyst 1989;189:627-30.
4. Eboka CJ, Aigbavboa SO, Akerele JO. Colorimetric determination of fluoroquinolones. J Antimicrob Chemother 1997;39:639-41.
5. Nagaralli BS, Seetharamappa J, Melwanki MB. Sensitive spectrophotometric methods for the determination of amoxicillin, ciprofloxacin and piroxicam in pure and pharmaceutical formulations. Pharm Biomed Anal 2002;29:859-64.
6. Lehto P, Kivisto KT, Pertti JN. The effect of ferrous sulphate on the absorption of norfloxacin, ciprofloxacin and ofloxacin. Br J Clin Pharmacol 1994;37:82-5.
7. Kara M, Hasinoff BB, Mckay DW, Campell NR. Clinical and chemical interactions between iron preparations and ciprofloxacin. Br J Clin Pharmacol 1991;31:257-61.
8. Kang JO, Jones C, Brothwell B. Toxicity associated with iron overload found in haemochromatosis: possible mechanism in a rat model. Clin Lab Sci 1998;11:350-4.
9. Schwartz KA, Fisher J, Adams ET. Morphologic investigations of the guinea pig model of iron overload. Toxicol Pathol 1993;21:311-20.
10. Chambers HF. Sulfonamides, trimethoprim and quinolones. In: Katzung BG, editor. Basic and Clinical Pharmacology. San Francisco, CA, USA: Lange Publication, 2007: 763-70.
11. FDA ALERT [7/8/2008]: (http: //www.fda.gov/medwatch/safety/2008/safety08.htm#Fluoroquinolone).