Bulletin of Faculty of Pharmacy, Cairo University xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bulletin of Faculty of Pharmacy, Cairo University

journal homepage: www.elsevier.com/locate/bfopcu

Original Article

Enhancement of antibacterial activity of ciprofloxacin hydrochloride by complexation with sodium cholate

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ARTICLE INFO

Keywords:
Ciprofloxacin
Sodium cholate
Ion-pair complex
Antibacterial activity
Enhanced activity

ABSTRACT

Ciprofloxacin is a broad spectrum bactericidal anti-infective agent of the fluoroquinolones class used in treatment of many bacterial infections. In recent times, there has been increasing resistance to the antibiotic. In this work, we investigated the effect of making an ion- pair complex of Ciprofloxacin – hydrochloride with Sodium cholate on bacterial activity. The optimal ratio of the reactants and pH were determined using UV spectrometry. The complex was characterized by octanol-water partitioning, melting point, and IR spectrometry. The antibacterial activity of the complex was determined against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *Streptococcus pneumoniae* by the agar-well diffusion method. The complex was whitish to off-white in color and crystalline, with a melting point of 238 °C. The stoichiometry of the complex shows a molar ratio of 1:1 of sodium cholate to ciprofloxacin. The best pH for complexation was pH 9. The complex partitioned 3.38 times into octanol than in water. The FTIR revealed interaction between the 4-nitrogen atom in the 7-piperazinyl group of ciprofloxacin and the carbonyl of the cholate. The drug in complex form gave double the antibacterial activity of the uncomplexed drug. This study showed that development of hydrophobic ion pair complex enhances antibacterial activity of ciprofloxacin hydrochloride.

1. Introduction

Ciprofloxacin hydrochloride is a fluorinated antibiotic used in the treatment of bacterial infections. Its antibacterial action is mainly by DNA gyrase and topoisomerase IV inhibition [1]. Ciprofloxacin proves to be effective in the treatment of infections caused by microorganisms like *Enterobacteriaceae, Escherichia coli, Vibrio, Haemophilus ducreyi, Haemophilus influenza, Neisseria gonorrhoeae, Neisseria meningitides, Mycobacterium intracellular, Moraxella catarrhalis, Brucella campylobacter, Bacillus anthracis, Pseudomonas aeroginosa* [2]. Hence, it is approved for the treatment of 14 types of infections, especially urinary tract infections such as acute uncomplicated cystitis; chronic bacterial prostatitis; gonorrhea; lower respiratory tract infections, bone-joint infections, typhoid fever, community acquired pneumonia, bacterial conjunctivitis, corneal ulcer, bronchitis, acute sinusitis, complicated pyelonephritis, inhalational anthrax, infection of skin or subcutaneous tissue and abdomen [3,4]. Also, research has shown that ciprofloxacin is efficacious in the treatment of chancroid caused by *Haemophilus ducreyi,* which is the major cause of genital ulcer disease in developing countries [5,6]. Ciprofloxacin is reported to have enhanced spectrum of activity as a result of the fluorine atom at position 6 and piperazine ring at position 7 [7].

A research conducted on the aqueous solubility of ciprofloxacin in the presence of metal cations showed that metal cations such as ferrous, ferric, calcium, aluminium, sodium and potassium ions increased ciprofloxacin solubility while magnesium sulphate decreased its solubility [8]. Some works carried out on complexes of ciprofloxacin showed significant improvement in drug development such as (i) Drug retention capability at retention site; (ii) Reduction in potential loss of expensive drug during the formulation process; (iii) Increase in the lipophilicity of the drugs thereby allowing delivery of larger amounts of the drug to infected cells [9–11].

Peer review under responsibility of Faculty of Pharmacy, Cairo University.
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http://dx.doi.org/10.1016/j.bfopcu.2017.09.006
Received 6 February 2016; Received in revised form 4 June 2017; Accepted 26 September 2017

A study involving hydrophobic ion-pair complexes of ciprofloxacin with sodium lauryl sulphate and sodium deoxycholate which were incorporated in the core of submicron-sized oil-in-water (o/w) emulsion showed improved payload efficiency and potential for prolonged retention with improved antimicrobial efficiency by imparting cationic charge on the surface, which can be used as potential drug delivery vehicle for topical and parenteral application [12]. Another study investigated the antimicrobial efficacy and pharmacokinetics profile of ciprofloxacin loaded oil-in-water (o/w) submicron emulsion. They developed hydrophobic ion-pair complex of ciprofloxacin with sodium deoxycholate together with cationic inducer chitosan; which showed two fold and four fold enhancement in antimicrobial efficacy and pharmacokinetic profile [11].

However, resistance to anti-infective agents is a growing challenge [13]. Resistance to ciprofloxacin and other fluoroquinolones has increased remarkably since their introduction for UTI treatment. As a result, the use of ciprofloxacin in the treatment of uncomplicated cervical and urethral gonorrhea is no longer effective in some countries due to resistance [14]. Many studies worldwide reported a clear increase in ciprofloxacin resistance. For instance, in China, from 1998 to 2002 the incidence of ciprofloxacin resistance increased progressively from 46.6% to 59.4% [15]. From 2003 to 2009 in Canada, there was an outbreak of ciprofloxacin-resistant *Salmonella* enteric Serovar kentucky, non-typhodial isolate [16]. A report showed that *Salmonella* enteric Serovar Kentucky, is the most common serotype isolated from chicken, and has emerged in humans although said to be a rare cause of infection in human [17].

In a study by Al-morzooq et al., ninety-three Malaysian extended-spectrum β-lactamase (ESBL)-producing *Klebsela pneumoniae* isolates were screened for ciprofloxacin resistance [18]. Here, 37% of the isolates showed resistance to ciprofloxacin with multiple mutations either in gyr A alone or in both gyr A and Par C region. Emerging global story of resistance to ciprofloxacin is a growing concern because it limits treatment option for invasion of disease. Moreover, poor aqueous solubility, systemic side effects and inadequate delivery system of ciprofloxacin has become a problem, suggesting development of new delivery system to treat bacterial infections that have become exceedingly unresponsive to standard antibacterial therapy and modification of existing drugs.

In this work, we developed a hydrophobic ion-pair complex of ciprofloxacin and sodium cholate in order to increase therapeutic efficacy and hopefully reduce adverse effects when dose is reduced.

2. Materials and methods

2.1. Preparation and characterization of ciprofloxacin-cholate complex

According to the method of Mishra [12], with modifications, 2 g of ciprofloxacin hydrochloride was weighed and reconstituted to 57.14 ml with distilled water while 2.233 g of sodium cholate was reconstituted to 57.2 ml with distilled water. Each was agitated properly. Sodium cholate solution in a beaker was added ciprofloxacin hydrochloride solution drop wise while stirring. When stirring became stiff, the pH was adjusted to 9. The mixture was filtered and the white precipitate was washed five times with distilled water to remove soluble impurities.

2.2. Determination of physico-chemical characteristics of ciprofloxacin and ciprofloxacin-cholate complex

2.2.1. Determination of pH

Three 0.2 g quantities of ciprofloxacin and ciprofloxacin-cholate complex were each introduced into separate beakers and dissolved in and dissolved in 30 ml of methanol. The volume was made up to 100 ml with water. The pH meter was used to determine the pH value.

2.2.2. Particle size, polydispersity index and zeta potential

A 100 mg sample was finely dispersed in 30 ml of a 1% tween 80 solution. A 20 µL volume of the freshly prepared suspension was diluted to 1000 µL with distilled water and the process was repeated. The diluted sample was now used for measurements in triplicates. The particle size, polydispersity index and zeta potential were determined using Zetasizer nano ZS (Malvern, UK).

2.2.3. Determination of free ciprofloxacin in ciprofloxacin-cholate complex

The absorbance of ciprofloxacin-cholate complex was determined at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mg/ml using UV-Vis spectrophotometer at 309 nm (λ_{max}) of ciprofloxacin hydrochloride. According to Bhalerao and Rote [19], using Beer Lambert, the values of standard calibration curve were used to calculate the amount of ciprofloxacin in the complex, given the formula as follows:

$$Concentration\ (unknown) = \frac{A\ (unknown) - Intercept}{Slope\ (standard)} \quad (2.1)$$

2.2.4. Determination of melting point

The melting point of the ciprofloxacin and ciprofloxacin-cholate complex was carried out using standard procedure. A small quantity of finely powdered drug sample (ciprofloxacin and ciprofloxacin-cholate) was packed separately into a thin-walled capillary tube which has first been sealed at one end. The capillary tubes and the thermometer were placed in appropriate apertures in the heating block. The temperature was raised uniformly at 10 °C intervals and the melting point was recorded [24,25].

2.2.5. Determination of solubility

Solubilities of ciprofloxacin hydrochloride and the complex were determined in water, methanol, ethanol, acetone, ethyl acetate, petroleum ether, chloroform, and hexane.

2.2.6. Octanol-water partition or distribution coefficient

This was determined using Shakeflask's Method. Aliquots of 50 mg, 100 mg and 150 mg of the drug complex were weighed and placed into three separatory funnels. A fifty milliliters volume of octanol was pipetted into each of the funnels, using a pipette filter. A stopper was used to close the end of the separator funnel and shaken well for 10 mins until all the sample had dissolved. Also, 50 ml of distilled water was added into the funnels and shaken vigorously for 10 mins. The separator funnel stood for 10 mins to allow separation of the two layers. Aqueous phase at the bottom of the separator funnel was run off gently into a beaker, while the octanol layer was run into another beaker. Ten (10) milliliters sample from the octanol layer was pipetted into conical flask and two drops of freshly prepared phenolphthalein solution was added and titrated with 0.2 M NaOH solution using phenolphthalein solution as indicator. Likewise, 10 ml portion from the lower aqueous layer was also titrated with 0.2 M NaOH. This was done 3 times and the average titer was taken. The partition coefficient was calculated as

Partition coefficient = volume of tritrant in octanol/volume of titrant in water

2.3. Fourier transform infra-red (FTIR) scan

A 2 g quantity of each of powdered sample was crushed in a Buck 530 IR mortar with 0.5 g of KBr, after which 2 ml of Nujol was introduced to form a paste before introducing it into the instrument sample mould and allowed to scan at wavenumber from 600 to 4000 cm^{-1} to obtain its spectrum.

2.4. Determination of ciprofloxacin-cholate complex stoichiometry

According to Job's method of continuous variation, an equi-molar solution of 10^{-4} M standard solution of ciprofloxacin hydrochloride