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In vitro food–drug interaction study: Which milk component has a decreasing effect on the bioavailability of ciprofloxacin?

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ABSTRACT

The purpose of the present work was developing an *in vitro* dissolution test to highlight the possible molecular background causing ciprofloxacin (CPFX)–milk interaction. The *in vitro* dissolution of CPFX from film-coated tablets (Ciprinol® 500 mg) was examined at different pH values, simulating certain parts of the gastrointestinal tract, in the presence of water, low-fat milk, casein- or calcium enriched water. In order to determine the amount of dissolved CPFX, solid phase extraction sample preparation followed by high performance liquid chromatography coupled with mass spectrometry was applied. Comparing the dissolution efficiency values in various media, it can be concluded, that casein has a more pronounced effect on the absorbable amount of the antibiotic at each pH value studied, than calcium. In the case of concomitant intake of CPFX film-coated tablet and milk or other dairy products not only the complexation with calcium, but also the adsorption of CPFX on the surface of proteins decreases the absorbable amount of CPFX.

1. Introduction

Fluoroquinolones (FQs) are a relatively new class of antibiotics widely used in human and veterinary medicine, with broad-spectrum activity against infections of the urinary tract, skin, soft tissues and respiratory tract, as well as against sexually transmitted diseases [1]. FQs (*e.g.* ciprofloxacin, ofloxacin, enrofloxacin, norfloxacin) act by inhibiting DNA gyrase, thus inhibiting bacterial DNA replication and transcription, culminating in rapid cell death [2,3]. Patients often take antibiotics with meals or dairy products to help swallowing them easier and to lessen their gastrointestinal side effects [4]. The current meal required by the US Food and Drug Administration (FDA) used to quantify drug–food interaction consists of two eggs fried in butter, two strips of bacon, two slices of toast with butter, four ounces of hash brown potatoes, and eight ounces of whole milk [5].

It is known, that food–drug interactions may occur by many mechanisms, and they can result in changes both in the rate and the extent of absorption [6–9].

Studies of the interaction between calcium and FQs have produced conflicting results. A 'standard breakfast' did not appear to impair the absorption of quinolones [10–12] and had only a minimal effect on their bioavailability – as it was observed with concomitant food administration of fleroxacin [13]. A further FQ–food interaction study did not result in any significant effect on CPFX's bioavailability [14], being consistent with another study where calcium carbonate capsules did not affect the absorption of CPFX [15]. Non-significant effect was observed, if CPFX was administered with a high-calcium-breakfast [16]. According to an FDA monograph, the bioavailability of CPFX did not alter significantly, if it was administered with orange juice, but it did alter, if orange juice was calcium-fortified, highlighting the potential role of calcium in the FQ–food interaction [17]. On the other hand, there are a number of studies, stating that the bioavailability of FQs is significantly influenced by selected alimentary components. According to *in vivo* data the gastrointestinal absorption of norfloxacin and CPFX was markedly decreased by the concomitant ingestion of milk or yoghurt [18,19]. Studying levofloxacin co-administered with mineral-fortified breakfast consisting of juice, cereal with or without milk, it could be concluded, that none of the studied fed phases (breakfast with or without milk) were bioequivalent to the fasting arm [20]. Similarly, concomitant administration of calcium carbonate tablets reduced the mean bioavailability of CPFX by 43% [21]. In an *in vitro* experiment on the release of CPFX over a cellulose membrane it was observed, that in the presence of calcium, aluminum or iron cations the CPFX release was slower than in the absence of the metal ions [22]. It can be stated that the circumstances of the above-mentioned studies (pH, food content, like fat, protein, calcium amount, etc.) were not the same, thus the results cannot be compared directly.

As all the examined alimentary components/meals contain large amounts of calcium an interaction with FQs seems to be likely.

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Because of their high-calcium content, milk and other dairy products may impair the gastrointestinal absorption of FQs, which form sparingly soluble chelates with di- and trivalent metal ions [23–29]. The pH of the gastric milieu may also be an important determinant of the magnitude of the interaction, since ionization of the carboxylic group of the quinolone molecule allows for more effective chelating with the cation [30].

The contradictory interaction results published in the literature indicate the limitations in the field of food–drug interaction [31], at the same time emphasizing the need of *in vitro* studies observing FQ-milk interaction. In the present work we aimed to develop an *in vitro* dissolution test to highlight the possible molecular background causing CPFX–milk interaction. The *in vitro* dissolution of CPFX from film-coated tablets (Ciprinol® 500 mg) was analyzed at three different pH values (pH 1.2, 4.5 and 6.8) simulating certain parts of the gastrointestinal tract in the presence of water, low-fat milk, casein- or calcium enriched water. The effect of these alimentary components was compared to the results obtained for the CPFX-dissolution in the appropriate aqueous medium. In order to determine the amount of dissoluted CPFX originating from various matrices of the dissolution test, a previously validated method – using solid phase extraction (SPE) sample preparation followed by high performance liquid chromatography coupled with mass spectrometry (HPLC–MS) – was used in our study [32].

2. Materials and methods

2.1. Reagents and chemicals

Acetonitrile, methanol and water originated from Sigma–Aldrich Ltd. (Steinheim, Germany) were gradient grade. Ammonium-acetate, calcium chloride hexahydrate, casein (technical grade), CPFX hydrochloride salt (98.0% by HPLC), formic acid, sodium hydroxide and trifluoroacetic acid (TFA) (99%) were purchased from Sigma–Aldrich Ltd. (Budapest, Hungary). Milk Quick® Instant low-fat milk powder was delivered by Instantpack Ltd. (Berettyóújfalu, Hungary). Calibration standards of milk analyses were kindly offered by the Hungarian Dairy Research Institute (Mosonmagyaróvár, Hungary). To clean and to set the zero point of Milko-Scan 130-Series milk analyzer system 0.1% Triton X-100 (Sigma–Aldrich Ltd., Germany) solution was used. Aripiprazole (ARI) – as internal standard – was obtained from LGC Promochem (Wesel, Germany). Potassium dihydrogen phosphate and hydrochloric acid (Sigma–Aldrich Ltd., Germany) were used to prepare dissolution media during the dissolution test of Ciprinol® 500 mg film-coated tablets (KRKA, Slovenia).

2.2. Dissolution test

In vitro food–drug interaction study on CPFX was carried out with a paddle apparatus (Hanson SR8-Plus Dissolution Test Station, Hanson Research Corp., Chatsworth, CA, USA) prescribed by the United States Pharmacopeia. The dissolution of CPFX from Ciprinol® 500 mg film-coated tablets was analyzed at three different pH values (pH 1.2, 4.5 and 6.8) – according to the pH conditions of the gastrointestinal tract – in the presence of various food or food components (low-fat milk, calcium, casein). The tests were conducted using 500 ml of dissolution medium. Dissolution media were prepared by the use of hydrochloric acid solution, potassium dihydrogen phosphate solution or potassium dihydrogen phosphate added to sodium hydroxide solution with the pH values 1.2, 4.5 and 6.8, respectively, according to Ph. Eur 5. CPFX containing film-coated tablet was dropped into the dissolution fluid thermostated at 37 ± 0.5 °C and at the same time 250 ml – 'one FDA glass' – of either water or calcium/casein enriched water or low-fat milk was added into the dissolution vessel. The stirring speed of the paddle was 50 rpm. Aliquots (1.00 ml) were taken at the following sampling times: 5, 10, 15, 30, 45, 60, 90 and 120 min from the release medium and were not replaced by equal volume of the receptor medium. All measurements were carried out in nine parallels; data are given as average ± S.D.

2.3. Dissolution efficiency (DE)

To characterize drug release profiles dissolution efficiency (DE) parameter was used [33] and defined as the area under the dissolution curve up to a certain time t, expressed as a percentage of the area of the rectangle arising from 100% dissolution in the same time. DE can be calculated by the following equation:

$$\mathrm{DE} = \int \frac{y\,\mathrm{d}t}{100t}$$

where y is the drug percent dissolved at time t. In this paper, all dissolution efficiencies were obtained with t equal to 120 min.

The areas under the curve (AUC) were calculated for each dissolution profile by the trapezoidal rule implemented on Microsoft Office Excel 2003 for Windows.

2.4. Sample preparation

2.4.1. Stock solutions

1.0 mg/ml stock solution of CPFX was prepared by dissolving 25.0 mg CPFX in 25.0 ml water; 5.0 mg ARI, as internal standard was dissolved in 50.0 ml methanol for the preparation of stock internal standard solution with a concentration of 0.1 mg/ml. SPE and HPLC–MS methods were developed and optimized using the above-mentioned stock solutions.

2.4.2. Low-fat milk

Low-fat milk was prepared by dispersing the milk powder in distilled water. Low-fat milky medium was prepared from 90.0 g milk powder and 850.0 ml distilled water according to the package instructions. The prepared milky media were stored for 24 h at +4 °C temperature in refrigerator, in order to get a homogeneous system.

2.4.3. Milk analyses

Because of the diversity between the traded milk goods, in order to standardize the biological matrix we used freeze-dried milk from the same batch dissolved in distilled water instead of fresh milk. Milk samples were analyzed with a Milko-Scan 130-Series (Type 10900, Foss Electric, Hillerod, Denmark) Flow System & Infrared System. Fat, protein and lactose – contents of low-fat milk, used as dissolution medium-components in the dissolution test – were analyzed by Milko-Scan 130. The determined fat, protein and lactose contents for low-fat milk were 0.12 ± 0.005 g/100 ml, 3.13 ± 0.1 g/100 ml and 4.91 ± 0.1 g/100 ml, respectively. Before analyses milk samples were preheated to 40 °C in water bath in order to get a homogenous system.

2.4.4. Calcium enriched water

Calcium enriched water was prepared to study the effect of milk calcium on the dissolution profile of CPFX. According to the USDA National Nutrient Database for Standard Reference [34] calcium content of milk was taken as 204 mg calcium in 100 g low-fat milk solution. Thus, 250 g calcium enriched water, containing the same amount of calcium as the same volume of low-fat milk, was prepared by adding 2.79 g calcium chloride hexahydrate (equivalent to 510 mg calcium) to the appropriate amount of distilled water.