Saudi Journal of Medical and Pharmaceutical Sciences
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2413-4929 (Print)
ISSN 2413-4910 (Online)

Intestinal Permeation of Piperaquine in the Presence of Ciprofloxacin after Drug Release from Dihydroartemisinin-Piperaquine Co-formulated Product

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Original Research Article

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Article History
Received: 16.09.2017
Accepted: 21.10.2017
Published: 30.10.2017

DOI:
10.21276/sjmps.2017.3.10.8

Abstract: Fluoroquinolone antibacterial agents are broad spectrum molecules and may be co-prescribed with antimalarial drugs in co-presenting infections. This study aimed at assessing the intestinal permeability of piperaquine (PQ) from dihydroartemisinin-piperaquine (DP) co-formulated antimalarial co-prescribed with ciprofloxacin (CP), using *ex vivo*absorption model. Excised intestinal segments (duodenum and ileum) of length 4 cm from New Zealand white albino male rabbits (n=2) loaded with DP suspension equivalent to PQ (100 mg/mL) and CP suspension (100 mg/mL) based on body weights of animals. Similarly, DP alone was loaded to serve as controls C1 and C2, respectively. The organ bath contained Tyrode solution (TS) 100 mL. Sampling (5mL) was taken at 0, 0.5, 1, 2, 4 and 6 h post immersion of tissue. PQ analysis was performed using high pressure chromatographic system with C8 ZorbactEclipse XDB (150 x 4.6 mm, 4.6 µm) column with UV detection at 220 nm and flow rate of 0.7 mL/min. Mobile phase contained acetonitrile: 10 mM ammonium acetate (70: 30 %v/v) and flow rate of 1.0 mL/min at ambient temperature. Area under the curve (AUC) ± SEM at 2 h and 6 h ($AUC0_{0-2}$ and AUC_{0-6}) for tests and their respective control in duodenum were (0.2940±0.1055 versus 0.6198±0.0083 µg.mLh^{-1}, P=0.009) and (1.9270±0.1287 versus 3.3975±0.3638 µg.mLh^{-1}, P=0.006) and ileum (1.5300±0.1242 versus 1.5408±0.4275 µg.mLh^{-1}, P=0.645) and (3.9500±0.0205 versus 5.6603±0.1073 µg.mLh^{-1}, P=0.045), respectively. CP revealed lower permeability indices for PQ in duodenal but not in ileal intestinal barrier. Spacing out of drug regimen may be required for optimum PQ permeation.

Keywords: Ciprofloxacin, Dihydroartemisinin-piperaquine, Piperaquine, Permeability, Duodenum, Ileum.

INTRODUCTION

The permeability of biological membranes is an important determinant of a drug's pharmacokinetic processes [1]. Many drug substances are transported across biochemical membranes by passive transcellular diffusion while most of membrane drug transport process involves Carrier-mediated mechanism [2]. Both mechanisms can co-exist based on evidence of physiochemical characteristics demonstrated by *in vitro* and *in vivo* findings [3].

The overall drug absorption of orally administered drug can be largely influenced by co-prescribed drug(s). The physiochemical and physiological influence of a drug can influence the bioavailability of drug of interest [4, 5]. The fundamental relationship between the rate of drug absorption when measured as a permeability coefficient has led to the employed experimental models as a surrogate for predicting the absorption of oral drugs [6]. There is the need to modify the available experimental models on drug disposition at absorptive sites to reflect real life situations (*e.g.*, in the presence of co-prescribed drug(s).

In recent times, major advances in the understanding of drug-drug interactions (DDI), especially with regard to the molecular mechanisms of interaction have been recorded [7]. In the light of the knowledge of DDI, improving the coverage, accuracy and application of DDI has been proposed to improve delivery of safe and cost-effective patient care [7]. The mechanisms and consequences of DDI will describe events as rare, minor and severe with the appropriate clinical caution observed. Drugs with narrow therapeutic index are likely candidates for [8]. Drugs in common use in that category include warfarin, fluoroquinolones agents, antiepileptics and oral contraceptives [9].

The antimalarial DP is a fixed dose combination (FDC), artemisinin-based combination therapy (ACT) approved by the World Health Organization for the treatment of multi-drug resistant uncomplicated infection [10]. The absorption of DP has

been well described in drug pre-formulation and formulation protocols [11-13]. However, co-prescribing of DP with antimalarial drugs, that are likely to combat co-existing infections, may alter the rates of drug absorption consequently impinging on the delivery of safe and cost effective patient care [13].

Ciprofloxacin, a fluoroquinolone antibacterial is often co-prescribed with DP but there is no documented evidence of molecular or biomembrane interactions in the literature with respect to these drugs. However, there are some mechanisms of DDI investigated onfluoroquinolone antibacterial agents with magnesium-aluminum antacids (*i.e.*, showing chelation of molecules) and with theophylline (*i.e.*, evidencing inhibition of metabolism) [14].

This study was aimed at assessing the effect of CP on PQ permeability from DP drug products traversing intestinal membrane using an *ex-vivo* permeability protocols.

EXPERIMENTAL

Materials

P-Alaxin® (DP) product of Bliss GVC, India and Ciprotab® (CP) product of Fidson Healthcare, Nigeria were purchased in a registered pharmacy, Lagos, Nigeria. Reference standard PQ and tinidazole powder were donations from Central Research Laboratory (CRL), University of Lagos, Nigeria. Sodium chloride, potassium chloride, calcium chloride, magnesium chloride, sodium bicarbonate, and sodium dihydrogen phosphate were analytical grade reagents products of Sigma Aldrich, Germany. Glucose was product of Evans Medical, Nigeria. Acetonitrile, methanol and ammonium acetate were of HPLC grade, products of Sigma Aldrich, Germany. Distilled water was used throughout the study.

Preparation of Standard Solution of Piperaquine/Internal Standard/Working solution

Stock solution of PQ was prepared by weighing 50mg of reference standard PQ and dissolved in 10 mL volumetric flask using TS. Graded concentrations (1-100 µg/mL) were prepared by serial dilution for calibration curve. Internal standard was prepared by dissolving accurately weighed tinidazole (50 µg) and dissolved in 5 mL of TS. The admixtures of drugs for loading were obtained by preparing DP solution equivalent to PQ (100 mg/mL) and CP solution(100mg/mL). The equivalent volume that delivered the required amount of PQ and CP based on the body weights of animals were drawn and mixed for loading in the excised intestinal segments.

Preparation of Experimental Animals

New Zealand white albino rabbits weighing 1.8 and 2.0 kg were used in the study. The animals were fed with standard pellet diet and water *ad libitum*, and were allowed to acclimatize for one week before the experiment started. Animals were fasted overnight prior to the experiment. Animals were paralyzed by cervical dislocation prior to surgical incision of the intestine. The protocol of the study was approved by Faculty of Pharmacy, University of Uyo Ethical Committee on the use of laboratory animal (UUFP012). Good laboratory practice was observed.

Experimental Design and Setup

The organ bath was set up using 100 mL of TS with a mechanical aerator. The isolated tissues were cut into sizes of 4 cm. The excised tissues were tied at one end with silk thread. The investigated drugs were loaded into the tissues before tying the other end and thereafter inserted into organ bath. Sampling (5 mL) was done at 0, 0.5, 1, 2, 4 and 6 h, with same volume replacement after each withdrawal. The obtained samples were filtered using 0.45 µm acrodysc syringe filter. Samples were stored until analysis in ultra freezer at -20 ^{0}C.

Sample Analysis

The analytical method was developed and validated by Central Research Laboratory (CRL), University of Lagos, Nigeria and was a modification of that developed by Deokate *et al* [15]. Standard solutions of PQ were prepared in methanol and run through HPLC to optimize analysis and standardize calibration. The chromatographic system (Chemstation, USA) operated at a flow rate of 0.7 mL/min and had in place a UV detector set at 220 nm. A C8 Zorbact Eclipse XDB (150x 4.6mm x 4.6 µm) column was used. The mobile phase was a mixture of acetonitrile: 10 mM ammonium acetate (70: 30%, v/v) and sample injection was 0.7 µL. The flow rate was set at 1.0 mL/min and the run time for each sample was8 min. All data were recorded manually and analyzed with computer multiplying with the appropriate dilution factors. APK for Pharmacokinetics software version 13 (Rxkinetics, USA) was employed to draw values for pharmacokinetic parameters.

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Scientist (SPSS) version 20 (IBM, USA). Paired T-test was used to compare the data for the test and the control groups. A p-value of less than 0.05 was considered to represent a statistically significant difference. All values are presented as mean±standard error of mean (SEM).

RESULTS

PQ was detectable in the TS and there were no interferences in the peaks with the IS. The correlation coefficient for the determination of PQ was R^2=0.999. Figure 1 showed a representative chromatograph of the

sample perfusate into the TS representing the systemic circulation. The AUC for PQ permeation through the duodenum in the presence of CP was significantly lower at 2 h and 6 h compared with their respective control (P=0000).

The effective permeability coefficient (P_{eff}) portrays the biopharmaceutical indicator across the intestinal barrier. Figure 2 presents the outcome of the tests and their respective controls in this experiment. The P_{eff} for disappearance of PQ from the intestinal lumen in both regions of the intestinal lumen studied revealed higher values than their respective P_{eff} appearance in the TS ($P<0.05$). While P_{eff} appearance was significantly higher in test than the control in duodenum set-up ($P<0.05$), there was no difference in the values comparing the test in ileum and its control.

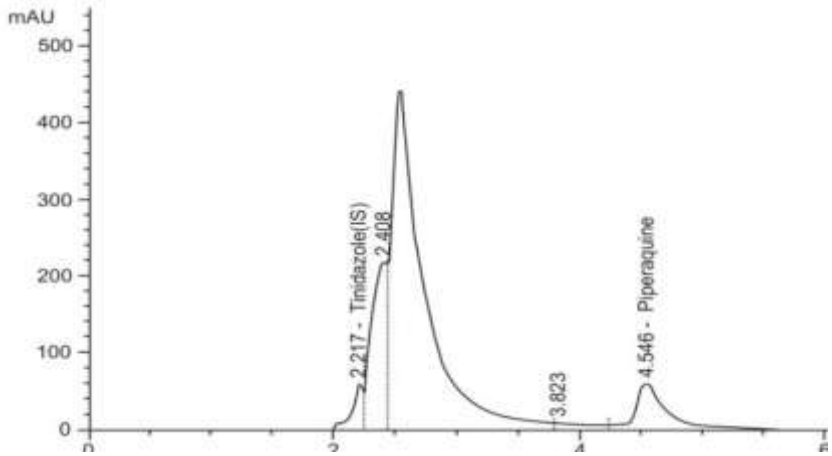

Fig-1: Representative chromatograph of sample of perfusate from the intestinal epithelium.

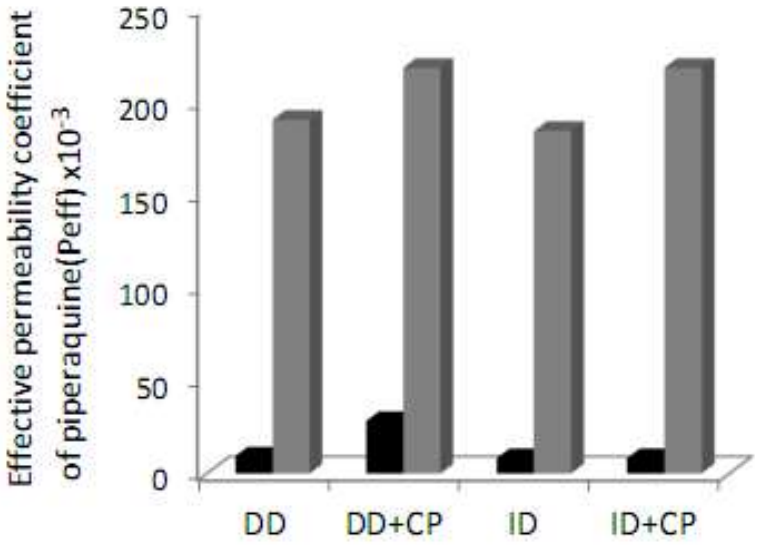

Fig-2: Effective permeability coefficient (P_{eff}) of piperaquine ■appearance, □disappearance, (DD = DP in duodenum, DD+CP = DP and CP in duodenum, ID = DP in ileum and ID +CP = DP and CP in ileum

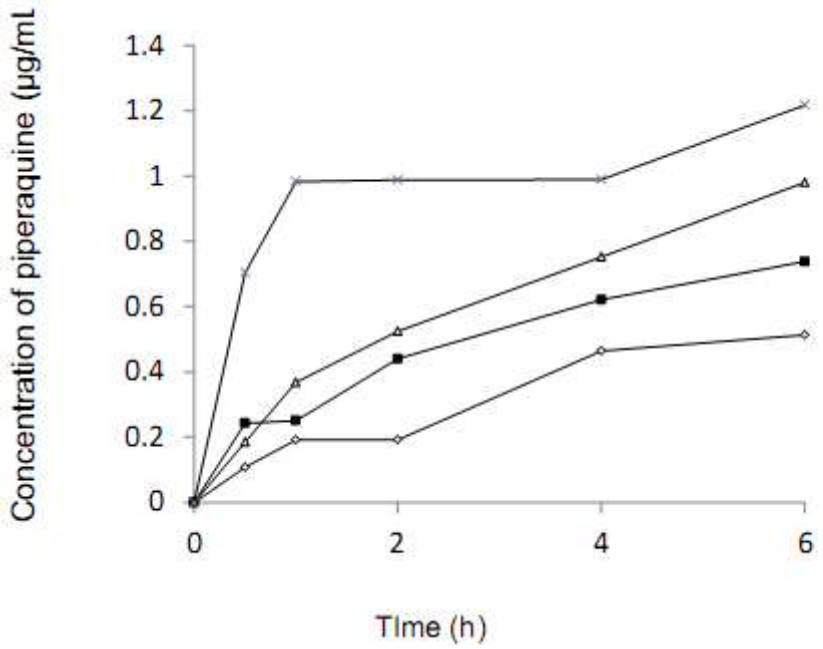

Fig-3: Piperaquine permeation in the presence of ciprofloxacin through duodenum (◊DDC), ileum (▄IDC) and their respective controls in duodenum (∆DD) and ileum (ˣID).

There was no significant difference in the rate constant(Ka)for PQ appearance in organ bath (0.0506±0.0066 versus 0.0317±0.0021 h^{-1}, P=0.098) and disappearance from ileal lumen (0.8712±0.0268 versus 0.7340±0.0072 h^{-1}, P=0.105) but higher value was observed for Ka appearance in organ bath and disappearance from duodenum (0.1112±0.0048 versus 0.0367±0.0008 h^{-1}, P=0.036) and (0.8778±0.0032 versus 0.7600±0.0008 h^{-1}, P=0.013).

Table 1: Kinetics of piperaquine release

Parameters		Media conditions			
		Duodenum		Ileum	
		Test	Control	Test	Control
Ka (h^{-1})		0.0070±0.0004	0.0023±0.0002	0.0020±0.0001	0.0020±0.0002
Cmax (µg/mL) ± SEM		0.5134±0.0038	0.9800±0.0025	0.7316±0.0452	1.2170 ±0.0070
R^2 values	Zero	0.9438	0.9297	0.5169	0.9087
	First	0.2497	0.0707	0.0047	0.1304
	Second	0.1776	0.2034	0.0135	0.2086
Kinetics of permeation		Zero	Zero	Zero	Zero

NB: Ka is the rate constant for permeation while Cmax is the maximum concentration of PQ achieved in the organ bath.

Table 2 presented the area under the curve (AUC) values for the tests conditions and their respective controls at 2 h (AUC_{0-2}) and 6 h (AUC_{0-6}). The AUC_{0-2} showed a lower value compared with the test (P=0.009) and similarly lower value for AUC_{0-6} test compared with control (P=0.006) in the duodenum. There was no significant difference in the AUC_{0-2} value for PQ permeation in the ileal segment (P=0.645) but a lower AUC_{0-6} value for test compared with the control (P=0.045).

Table 2: AUC values for the intestinal regions

Media condition	AUC $_{0-2}$ (µghmL^{-1})		AUC $_{0-6}$ (µghmL^{-1})	
	Duodenum	Ileum	Duodenum	Ileum
Test	0.2940± 0.1055	1.5300±0.1242	1.9270±0.1287	3.9500±0.0205
Control	0.6198±0.0083	1.5408±0.4275	3.3975±0.3638	5.6603±0.1073

NB: Determinations were performed in duplicates (i.e., n=2)

DISCUSSION

In the recent past years, major advances in the understanding of *in- vitro* absorption model, either cell or tissue-based set-up, have extensively demonstrated the relevance of this approach in the assessment of the intestinal permeability and absorption of drug substances in early developmental stages and post-marketing assessments for drug performance [16, 17]. By extension, the performance of a particular drug when co-administered with another drug can be assessed based on pharmacokinetic interaction evaluation, especially when taken orally. Possible drug-drug interaction (DDI) occasioned by the co-prescribing of DP with CP, a fluoroquinolone antibacterial agent, at the absorptive media of the intestinal region was studied in this work.

Previous researchers have highlighted the place of the physiochemical properties of a particular drug on its intestinal absorption with due consideration to prevailing physiological parameters [18, 19]. The presence of other molecular entities, such as CP, that may be co-prescribed with antimalarial agents such as DP in co-infection scenario involving malaria with another infection (*e.g*., typhoid fever) will require systematic evaluation for possible DDI that may jeopardize efficacy and safety of the employed drugs. Unanticipated drug interactions on co-prescribing of anti-infective agents may lead to altered bioavailability which may present a sub-therapeutic plasma concentrations and consequent treatment failure [20].

Some members of fluoroquinolone antibacterial agents (*e.g*., moxifloxacin and delafloxacin) have recently been noted with biliary excretion and transepithelial intestinal secretory activities in their description of CP membrane interaction [21, 22]. In this study, the permeation of PQ through the intestinal membrane in the presence of CP was investigated in the light of CP membrane interaction [23].

Towards the safe and efficacious use of DP, the WHO has made a publication on assessment and monitoring of antimalarial drug efficacy [24]. Our own work here seeks to explain the cellular basis of the quantitative difference in PQ permeation due to co-administration with CP. Also to provide a possible biochemical insight to the mechanism of the observed DDI in this drug combination [25].

The P_{eff} is one of the key biopharmaceutical parameters that determine the rate and extent of intestinal drug absorption. This parameter was derived from Fick's law and it relates the net flux (*i.e*., mass per unit area per unit time) through a membrane wall and the drug concentration across the membrane surface [26, 27]. The observed difference in the P_{eff} appearance and P_{eff} disappearance values for PQ permeation across the duodenal epithelia may be associated with the hydrophilic nature of CP and the consequent carrier-mediated transport across intestinal barrier. PQ traverses the membrane transcellularly being a lipophilic molecule while CP passes paracellularly as a hydrophilic drug [28]. The mechanism of CP intestinal efflux into the mucosal segmental and invariably into the lumen is poorly understood. The active efflux of CP has been reported to be highest at the ileocaecal region [3, 29].Just as the activity of the efflux transporter (PgP) has been reported to increase from the duodenum towards the colon (29, 30), it is expected that regional differences in the absorption or permeability of molecules will be observed. We speculate that the regional difference in the efflux of CP may influence the uptake of PQ, even though PQ is known to be a non-substrate for Pgp activities and therefore not involved in this bidirectional transport [31, 32]. This reverse absorption of CP against its concentration gradient may influence the uptake of PQ, a co existing molecule in solution in the intestinal lumen, in the opposing direction [32]. There is also a thermodynamic support of this proposal from CP active transport forming the basis of this antiport concept (simultaneous movement of different molecules in different direction) in favour of PQ passive diffusion, especially in the ileal segment [33, 34].

Some earlier researchers sought to know the likely inhibitors to CP intestinal efflux and reported that verapamil, benzbromanone or quinidine, on introducing to the mucosal side, did not interfere or inhibit CP efflux. They however revealed that introducing the same agents to the serosal side led to a significant inhibition of CP efflux[35]**.** It can therefore be deduced that the presence of PQ which is structurally related to quinidine both in the class of quinoline antimalarial agents may have similar membrane related influence on CP and *vice versa* [36].

In a study conducted by Hirano *et al*, CP was reported to exhibit a pH –dependent interference with the absorption of a structurally similar molecule, enoxacin [37, 38]. The authors reported that CP caused a reduction in the initial binding of enoxacin to the membrane surface and in the same vein reduced the K^+ and H^+potential-dependent transport across the rat jejunal membrane [39]. The work of these earlier researchers on electrochemical potentials provides insight to the possible influence of CP on membrane transport which will influence a co-administered agent's uptake. Electrochemical potential gradients have been reported to drive simple diffusion in the direction that eliminates the existing concentration gradient [39].Some drugs such as 14-membered macrolide antibiotics are known to produce alterations in digestive tract motor activity via production of strong gastric

contractions and also decrease small intestinal motility [40]. This drug interaction is considered a physiological based influence and requires deeper investigation into the salient molecular basis of CP influence on PQ permeability.

CONCLUSION

The co-loading of DP with CP caused a reduction in the rate and extent of PQ permeability in the duodenal epithelia but no significant effect on the ileal tissues. Further study is required to ascertain the net influence of CP on PQ permeability following oral co-administration of the drugs.

ACKNOWLEDGEMENT

This research was funded by TETFUND Research Fund 2015-2016 merged intervention. The authors wish to express their appreciation to Peter D. Ojobor of Central Research Laboratory, University of Lagos, Nigeria for the technical assistance and Ugochukwu I. Okoye and UkemeUko for their laboratory support.

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