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Original Research Article

Effect of Metronidazole on Piperaquine Permeability from Dihydroartemisinin-Piperaquine Antimalarial Product across Intestinal Membranes

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Abstract: The effects of metronidazole (MN) on intestinal absorption properties are less investigated. This work aimed at assessing the effect of MN on piperaquine (PQ) permeability from dihydroartemisinin-piperaquine (DP) co-formulated antimalarial product, across intestinal epithelial membrane. Excised intestinal tissues from New Zealand male albino rabbits (n=2) were loaded with DP equivalent to PQ (100 mg/mL) and MN (100 mg/mL), according to animals' body weight. Tissues were submerged in tyrode solution (TS) in an organ bath (100 mL). DP alone was similarly loaded in duodenum and ileum as control C1 and C2, respectively. Sampling (5 mL) of TS was taken at 0, 0.5, 1, 2, 4 and 6 h post immersion. Analysis of samples was performed using high pressure liquid chromatographic (HPLC) system with Zorbact Eclipse XDB column C8 (150 x 4.6 mm, 4.6 µm), mobile phase containing acetonitrile: 10 mM ammonium acetate (70:30, % v/v). The UV wavelength of detection and flow rate were 220 nm and 0.7 mL/min, respectively. The kinetics of PQ permeation was unaffected by MN. The area under the curve at 2 h (AUC_{0-2}) and 6 h (AUC_{0-6}) for duodenum revealed no difference (0.6285±0.0085 versus 0.6198±0.0083 µg.mLh^{-1}, P=0.500) but lower (2.4863±0.0328 versus 3.3975±0.3638 µg.mLh^{-1}, P=0.008) and for ileum, lower (0.1600±0.0170 versus 1.5408±0.4275 µg.mLh^{-1}, P=0.001) and (0.9460±0.0506 versus 5.6603±0.1073 µg.mLh^{-1}, P=0.011), respectively. The maximum concentration achieved were also lower than the respective controls (P<0.05). MN reduced the permeability of PQ across the intestinal regions. These findings will help to optimize therapeutic implications on concurrent administration.
Keywords: Dihydroartemisinin-piperaquine, metronidazole, piperaquine, intestinal permeability.

INTRODUCTION

Dihydroartemisinin-piperaquine (DP) is an antimalarial drug prescribed for multi-drug resistant uncomplicated *Plasmodium falciparum* malaria [1, 2]. The absorption profile of DP as a lipophilic-based drug combination has been reported to possess enhanced bioavailability (BA) when taken concurrently with fatty meals [3, 4]. Similarly, co-administration of metronidazole (MN)with some drugs has been reported to increase drugs' effectiveness. Examples of such drug interactions are observed with warfarin [5], clotrimazole [6] and vancomycin [7].

DP may be co-prescribed with MN, a commonly employed drug for intestinal infections due to protozoa (*e.g.*, E*ntamoeba histolytica*). The co-administration of DP and MN may also be incidental to co-existing intestinal infection with malaria, especially in resource-limited settings [8]. However, MN has been reported to trigger debilitating adverse reaction in alcoholics and patients with compromised liver function [9, 10].

Several drug-drug interactions (DDIs) have been reported following inappropriate prescribing or self medication [11]. In particular, interactions of rifampicin with non-steroidal anti- inflammatory drugs presenting as precipitants drugs are commonly encountered in primary care practice [12, 13]. The fundamental correlation between drug absorption rates and ingestion of medicines for co-existing health challenges has emerged into the use of experimental models as surrogate for predicting the oral absorption of drugs [14]. This has also presented a platform for a mechanistic assessment, at molecular level, the effects on co-administration of any drug, on the absorption of the other[15, 16].

A study reported on the potentiation of the anticoagulant effect of warfarin on co-administration with MN in healthy subject [17].Another concluded that MN lacks any safety concerns on single and multiple dosing regimens administration ceftazidime and avibactam [18, 19]. The reliability of outcome from

drug permeability experiments depends on choice of animal species, tissue source, cell line and the manner with which permeability experiments are conducted [20]. *Ex vivo* absorption model is useful tool for exploiting the regional differences in drug intestinal absorption, transport pathways and metabolism.

This study was aimed at assessing the effect of concurrent administration of MN with DP following up on PQ intestinal absorption.

EXPERIMENTAL

Materials

Piperaquine and tinidazole as internal standard (IS) reference powder were donated by Central Research Laboratory (CRL), University of Lagos, Nigeria. The investigated drug DP branded P-Alaxin® product of BVS, India and MN branded Flagyl® product of May and Baker Plc, Nigeria, were purchased from registered pharmaceutical outfit in Lagos, Nigeria. Potassium chloride, calcium chloride, sodium bicarbonate, sodium dihydrogen phosphate and sodium chloride were product of Sigma Aldrich, Germany. Acetonitrile, methanol and ammonium acetate were HPLC grade, products of Sigma Aldrich, Germany. Distilled water was used in this study.

METHODS

Preparation of Stock and Drug Solutions

Stock solution (5 mg/mL) was prepared by dissolving accurately weighed reference standard piperaquine (50 mg) in 10mL volumetric flask. The IS was spiked into the working standard solutions to give to 5µg/mL in each solution. A total of 5 tablets each of the investigated drug products (*i.e.*, DP and MN) were weighed simultaneously and their respective average weights deduced. The equivalent weights of the labeled active ingredients were considered to calculate the amount required to give a concentration of 100 mg/mL of actives in 10 mL dissolving in TS. The volumes of DP and MN solutions required for dosing were calculated based on 9.14 mg PQ and 5.74 mg of MN per kg body weight of rabbits, and mixed to obtain the required admixture solution.

Handling of Animals

The species of New Zealand White Albino male rabbit employed in this study weighed between 1.8 and 2.0 kg. Animals were fed with standard pellet diet and allowed access to water *ad libitum*. A period of one week was allowed to acclimatize [21]. Animals were fasted overnight but allowed access to water prior to the experiment. They were paralyzed by cervical dislocation before surgical exposure of the abdomen and excision of the intestine to isolate the intestinal segments. The protocol of the study was approved by Faculty of Pharmacy, University of Uyo Ethical Committee on the Use of Laboratory Animals (UUFP012). Good Laboratory Practice was observed.

Loading of Intestinal Compartment and Organ Bath Setup

Organ bath used was set up using 100 mL of TS with a mechanical aerator in place. The excised tissues were cut into approximately 4 cm length and tied at one end while loading with the admixture solutions of the investigated drugs based on calculated animal body weights. Similarly, DP alone was loaded in duodenum (C_1) and ileum (C_2) as controls. Sampling (5 mL) was performed at 0, 0.5, 1, 2, 4 and 6 h post immersion of loaded excised tissue in organ bath, with equal volume replacement after each sampling.

Sample Analysis

The method of analysis was developed and validated by Central Research Laboratory (CRL), University of Lagos and was a modification of the method by Deokate and co-workers [22]. Analysis was performed with reverse phase high pressure liquid chromatography (RP-HPLC) Chemstation with Zorbact Eclipse XDB C8 (150 x 4.6 mm x 4.6 µm) column. The mobile phase was a mixture of acetonitrile and 10mM ammonium acetate (70: 30, $\%^{v}/_{v}$) with UV wavelength of detection and flow rate set 220 nm and 0.7 mL/min, respectively. One microlitre of sample was injected to the port of the chromatographic system and the chromatogram analyzed.

Pharmacokinetic and Statistical Analysis

Every sample injection resulted in peak areas and mean peak area deduced. Mean peak area ratio from the IS peak was used to obtain a calibration curve relating the mean peak area ratio with PQ concentration. PQ concentrations versus time were imputed into APK Pharmacokinetic Software Version 13, (Rxkinetics, USA) and Microsoft Excel Version 7, (USA) to analyze the drug disposition and mechanisms of drug permeation. Statistical analysis was performed with SPSS Version 20 (IBM Company, USA). Results were expressed as the mean ±SEM. Paired T-test was used to compare the means for treatments at the two regions of the intestine.

RESULTS

The *ex vivo* absorption model was useful for assessing intestinal permeation of drugs and has revealed new effect of MN on intestinal membrane permeability. Figure 1 gives the sample of chromatogram for samples of PQ perfusate across intestinal epithelia. The concentration time curves for PQ permeation through the epithelial wall into the TS solution for test and control are similarly presented in Figure 2. The pharmacokinetic profile for PQ in the presence of MN revealed lower profile compared with the control for both segments of the intestine ($P<0.05$).

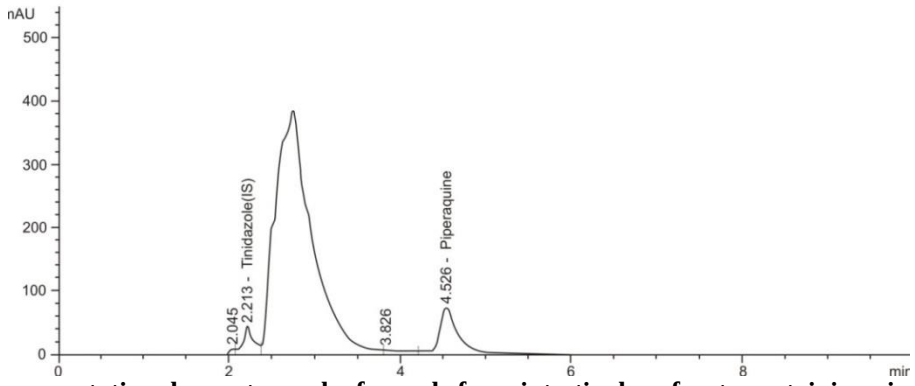

Fig-1: Representative chromatograph of sample from intestinal perfusate containing piperaquine

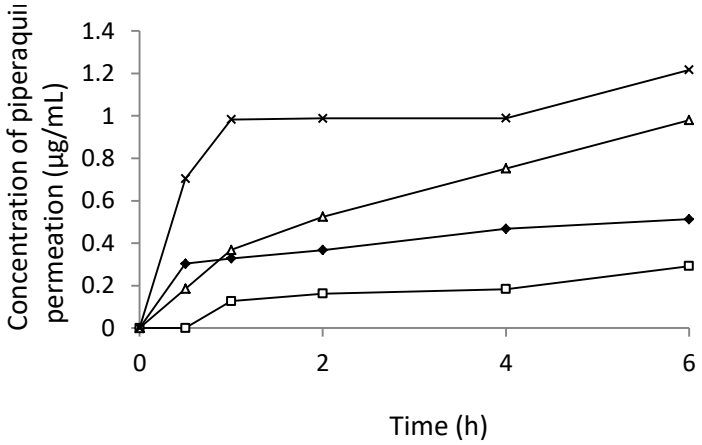

Fig-2: PQ permeation through duodenum and ileum in the intestinal epithelium (x ID = DP alone in ileum, □IDM = DP with MN in ileum, ∆DD = DP alone in duodenum and ♦DDM = DP with MN in duodenum

The effective permeability coefficient (P_{eff}) of PQ for appearance in the organ bath versus disappearance from the intestinal lumen is presented in Figure 3. The P_{eff} for disappearance of PQ from the duodenum/ileum presented higher values compared with the test. There was no significant difference in the P_{eff} disappearance for PQ in the regions (duodenum or ileum). Similarly there was no significant difference in the P_{eff} appearance in organ bath. The presence of MN revealed significant differences in the P_{eff} appearance and disappearance of PQ in the organ bath and from the intestinal lumen, respectively.

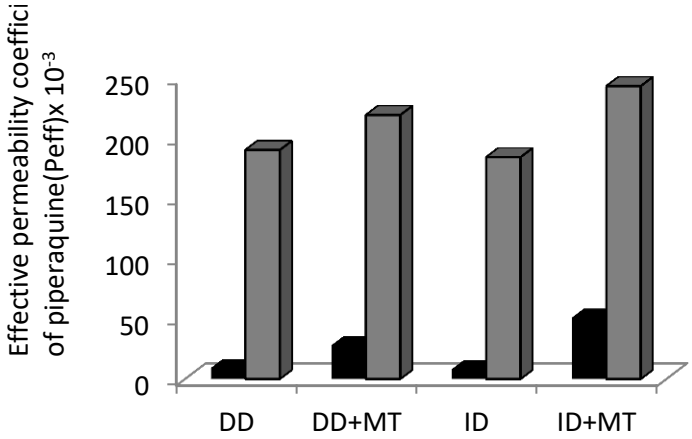

Fig-3: Effective permeability coefficients (P_{eff}) of piperaquine ■ appearance and □ disappearance, (DD = DP alone in duodenum, ID= DP alone in ileum, DD+MT =MN with DP in duodenum and ID+MN= MN with DP in ileum

There was no difference in the AUC_{0-2} for PQ permeation through duodenum in the presence of MN but a significant difference was observed for PQ permeation in ileum (P=0.001). Table 1 revealed the AUC_{0-2} and AUC_{0-6} for PQ permeation through the intestinal regions with significantly lower values compared with their respective control values.

Table 1: AUC measurements for the intestinal regions

Media condition	AUC 0-2 h (µg.hmL-1)		AUC 0-6 h (µg.hmL-1)	
	Duodenum	Ileum	Duodenum	Ileum
Test	0.6285±0.0085	0.1600±0.0170	2.4863±0.0328	0.9460±0.0506
Control	0.6198±0.0083	1.5408±0.4275	3.3975±0.3638	5.6603±0.1073

Table 2 expresses the maximum concentration achievable for PQ permeation though the intestinal epithelial for the test and control. Comparing C1 and C2 (*i.e.* duodenum and ileum), there was significantly higher PQ permeation in C1 than C2 (P = 0.007). MN caused significantly lower Cmax in duodenum (P=0.003) and ileum (P=0.001). The kinetics of PQ permeation was however not altered by the presence of MN in the intestinal regions.

Table 2: Permeation kinetics for piperaquine from the regional intestinal epithelia

Parameters		Media conditions			
		Duodenum		Ileum	
		Control	Test	Control	Test
(Cmax) ± SEM		0.9800±0.0025	0.5135±0.0579	1.217±0.007	0.2925±0.0006
R^2 values	Zero	0.9438	0.6645	0.5169	0.8618
	First	0.2497	0.0005	0.0047	0.1825
	Second	0.1776	0.9298	0.0135	0.0011
Kinetics of permeation		Zero	Second	Zero	Zero

NB: Cmax is Maximum Concentration achieved in TS

DISCUSSION

In vitro methods have, in recent years, advanced in their physiological relevance probing into mechanisms of drug absorption in gastrointestinal (GI) tract and evaluating DDI between co-administered orally absorbed drugs [23, 24]. Investigations on the delivery of systemically acting drugs across the intestinal barriers is useful in developing strategies for optimizing the amount of drug absorbed from products intended for oral use, especially as co-prescribing of medication is quite common. This present study assesses the transcellular PQ permeation from DP co-formulated antimalarial product when co-administered with MN.

DP on single administration has been reported to produce satisfactory plasma levels that give optimal parasitic clearance [25]. However there is little information in the literature on the DDI involving DP or its component actives on co-administration with commonly co-prescribed drugs. In this study, MN did not cause any significant difference in the permeation of PQ across the duodenal epithelium. However a significantly lower difference was observed in the ileum. Duodenum presents a larger surface area available for absorption, coupled with the lower pH presented for drug absorption. pH difference in the compartments have been reported as a major factor determining the extent of absorption of drugs [26].The pH in the duodenum ranges from 5-6 (slightly acidic hence PQ exists more as ionized molecules according to the pH partition theory [26]. The ileum with pH 7-8 units will present PQ, molecules as unionized, therefore facilitating higher bioavailability indices [27]. Molecules presenting more as unionized forms are able to sufficiently diffuse across biological membranes.

PQ is a highly lipophilic bisquinoline compound with a large apparent volume of distribution. Its absorption across the intestinal barriers therefore relates to its inherent capability to traverse biological membranes, as observed from its distribution potential [28]. Multiple intestinal transporters located on the brush border and basolateral membranes of the enterocytes have been implicated for drug absorption [29]. The potential for the involvement of any of these in PQ transmembrane absorption and possible competitive inhibition by MN will require future evaluation, as this can contribute to the mechanisms by which interaction may occur [29].

PQ is not transported by Permeability-glycoprotein (P-gp) ATP dependent transporter that is known to influence the passage of many antimalarial drugs across intestinal barriers [27]. PQ is not a substrate for P-gp nor is it an inhibitor, therefore the passage of PQ across intestinal epithelium is solely a diffusion process [28]. PQ presents a physicochemical

profile based on its lipophilic and basic nature and is expected to observe a transcellular passive diffusion across the absorptive membrane [29]. The observed P_{eff} appearance values for the test and control explains the basis for the biopharmaceutical implications of the co-administered drugs. Where the other factors are in consideration for the permeation mechanisms, the physiochemical properties of drug cum physiological factors (*i.e*., pH of environment) can account for the regional differences in PQ permeation across intestinal membrane [30].

A previous study by Awofisayo *et al* on the chemical interaction of DP with MN revealed that there was no chemical interaction between the investigated drugs on evaluating with Fourier transforms infra red (FTIR) spectroscopic methods [31]. Therefore the outcome in this study, following up on PQ permeation, was not influenced by chemical interaction between the investigated drugs.

The outcome of this tissue-based *ex vivo* model for intestinal permeability assessment therefore gives the basis for careful evaluation of co-prescribing of DP with MN as this may present some biopharmaceutical implications.

CONCLUSION

MN caused a significant reduction in the permeation of PQ across the intestinal epithelial on co-administration via the oral route following up with the *ex vivo* absorption model. Co-prescribing and oral administration of DP with MN may present biopharmaceutical implications, especially at a time that sub-optimal plasma concentration of actives in antimalarial products are established causes of fast development of parasitic resistance to drugs.

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